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CERTIFICATION REPORT

Certification of Equivalent Spherical Diameters of Silica Nanoparticles in Water

Certified Reference Material ERM[®]-FD100

EUR 24620 EN – 2011

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CERTIFICATION REPORT

Certification of Equivalent Spherical Diameters of Silica Nanoparticles in Water

Certified Reference Material ERM[®]-FD100

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Abstract

This report describes the certification of the equivalent spherical diameters of silica nanoparticles suspended in aqueous solution, Certified Reference Material (CRM) ERM-FD100[®]. The CRM has been certified by the European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE.

The intended use of this ERM-FD100 is to check the performance of instruments and methods that determine the particle diameter of nanoparticles (particle size ranging from approximately 1 nm to approximately 100 nm) suspended in a liquid medium. It is available in 10 mL pre-scored amber glass ampoules containing approximately 9 mL of suspension.

The CRM was prepared from commercially available colloidal silica (Koestrosol 1530, Chemiewerk Bad Koestritz GmbH, DE).

Certification of the CRM included testing of the homogeneity and stability of the ampouled diluted raw material, as well as the characterisation using an intercomparison approach.

The material has been certified for the equivalent diameter of the silica nanoparticles in aqueous suspension using different methods. Certified values are the cumulants dynamic light scattering (DLS) intensity-weighted harmonic mean particle diameter, the line-start centrifugal liquid sedimentation (CLS) intensity-based modal (Stokes) particle diameter, the electron microscopic (transmission electron microscopy (TEM)/ scanning electron microscopy (SEM)) number-based modal particle diameter and the small angle X-ray scattering (SAXS) intensity-weighted average particle diameter.

Indicative values have been established for the volume-weighted mean equivalent spherical diameter via the SAXS method and for the zeta potential via the electrophoretic mobility (ELM) method. Additional informational values are given for the volume-weighted mean diameter via the DLS method, and the pH value of the ERM-FD100 suspension.

Uncertainties are expanded uncertainties estimated in accordance with the *Guide to the expression of uncertainty in measurement* (GUM) with a coverage factor of $k = 2$, corresponding to a confidence interval of about 95 %. An exception is the mean equivalent volume-weighted diameter determined by the SAXS method which has a coverage factor of 2.8.

The following values were assigned.

Certified values

Equivalent spherical diameter/method	Certified value $\pm U_{\text{CRM}}$ [nm]
Intensity-weighted harmonic mean as determined by DLS cumulants method (ISO 22412:2008)	19.0 \pm 0.6
Intensity-based modal Stokes as determined by CLS line-start method (ISO 13318-1:2001)	20.1 \pm 1.3
Number-based modal as determined by EM (TEM/SEM) method (ISO 13322-1:2004)	19.4 \pm 1.3
Intensity-weighted mean as determined by SAXS	21.8 \pm 0.7

Indicative values

Equivalent spherical diameter/method	Indicative value $\pm U_{\text{CRM}}$ [nm]
Volume-weighted mean as determined by SAXS method	20.4 \pm 1.6

Measurand/ Method	Indicative value $\pm U_{\text{CRM}}$ [mV]
Zeta potential as determined by ELM method	-43.0 \pm 21.8

Additional material information

Equivalent spherical diameter/method	Additional value [nm]
Volume-weighted mean as determined by DLS method	16.9

Measurand /method	Additional value
pH as determined by potentiometric method with a glass electrode	9.7

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Glossary

ANOVA	Analysis of variance
CI	Confidence interval
CLS	Centrifugal liquid sedimentation
CRM	Certified reference material
d_{SAXS}	SAXS particle diameter
DLS	Dynamic light scattering
ELM	Electrophoretic mobility
EM	Electron microscopy
ERM[®]	European Reference Materials
ILC	Interlaboratory comparison
IRMM	Institute for Reference Materials and Measurements
ISO	International Organization for Standardization
JRC	Joint Research Centre
LTS	Long-term Stability
MSB	Mean square between groups from an ANOVA
MSW	Mean square within groups from an ANOVA
m/m	Mass fraction
n	Number of replicates
p	Number of data sets
PI	Polydispersity index
QCM	Quality control material
r_{Guinier}	Guinier radius
SAXS	Small angle X-ray scattering
SEM	Scanning electron microscopy
SI	International System of Units
STS	Short-term stability
s	Standard deviation of dataset means in the characterisation study
s_{bb}	Between-bottle (=ampoule) variability
s_{between}	Standard deviation between-groups (ANOVA)
s_{wb}	Standard deviation within bottle (=ampoule)
s_{within}	Standard deviation within groups (ANOVA)
t	t-factor

TEM	Transmission electron microscopy
<i>u</i>	Standard uncertainty
<i>u</i>_{bb}[*]	Between-bottle (=ampoule) heterogeneity that could be hidden by method repeatability
<i>u</i>_{bb}	Uncertainty related to a possible between-bottle (=ampoule) heterogeneity
<i>u</i>_{CRM}	Combined uncertainty of the certified value
<i>u</i>_{char}	Uncertainty of the characterisation
<i>U</i>_{CRM}	Expanded uncertainty of a certified value
<i>u</i>_{lts}	Uncertainty of long-term stability
<i>u</i>_{sts}	Uncertainty of short-term stability
<i>v/v</i>	Volume fraction

1. Introduction

Nanoparticles are particles with external dimensions between 1 nm and 100 nm [1]. Nanoparticles may exhibit unique properties due to their size. In order to understand the different properties of nanoparticles, reliable size measurements are needed. In this respect, appropriate reference materials, including quality control and calibration materials, are important [2].

Size and size distribution measurements are indispensable to understand the relevant nanoparticle properties. A variety of techniques exists to analyse the size and size distribution of nanoparticles in a suspension. Different techniques may result in different particle diameters due to the different measurement principles used for establishing the particle diameter. Therefore, discrepancy of results obtained with different sizing techniques is to be expected [3].

In addition, even using one and the same technique and despite expressing exactly the same physical property of a sample, the reported results can be quite different depending on the shape of the particles' distribution and the way it is transformed mathematically into an average value. Therefore, the certified particle diameter of the ERM-FD100 is specified in this report as an equivalent spherical diameter corresponding to the measurement method and evaluation approach used.

DLS measures the fluctuation of light that is scattered by a quiescent particle suspension. The fluctuation is due to on-going changes of the particle's positions by Brownian motion and can be related to the particle diffusion coefficient and its hydrodynamic diameter, respectively. DLS instruments analysing the intensity fluctuations in the time domain are using the cumulants analysis method. The signal fluctuation is measured via a frequency analysis method by DLS instruments that operate in the frequency domain [4].

The CLS method determines the modal Stokes diameter of suspended nanoparticles by measuring the velocity of the moving particles during application of a centrifugal force. Depending on their size and density, particles will sediment at different velocities. The velocity of sedimentation decreases with decreasing the size for particles of equal density [3]. In the line-start CLS method, a small volume of a dilute suspension of particles is injected into the centre of a spinning disc. The spinning disc chamber is filled with a liquid (e.g. sucrose solution) that has a slight density gradient, so that the liquid at the outside edge of

the ring is slightly denser than the one near the inside edge. The velocity of particles moving in a suspending density gradient medium under the action of an increasing gravitational field due to the rotation of the disc is measured. In the homogeneous CLS method (also called AUC or Photocentrifuge), the disc is replaced with a rectangular cell or cuvette containing the particle suspension.

SEM is a method that uses a beam of electrons, accelerated to high energy and focused on the sample, to image the sample surface. The sample is a substrate covered with well-dispersed nanoparticles for particle imaging and analysis. The focused electron beam generates secondary and backscattered electrons and X-rays that will allow one to obtain topographical and chemical information about the sample. In a SEM, the focused electron beam interacts with the sample at the surface whereas in a TEM, the electrons travel through the sample thickness. Particle size distributions are calculated from selected images. The SEM/TEM images are analyzed using Image-software to evaluate the individual nanoparticle sizes and particle size distributions [5].

In a SAXS experiment the sample is penetrated by an X-ray beam (transmission mode). The internal structure of the sample causes scattering of X-rays into all directions. The observation angle at which the scattered intensity is detected is called the “scattering angle” 2θ and is the angle between incident and scattered beam. Scattering vector is defined in terms of the scattering angle θ and the wavelength λ of the radiation. The scattering curve, meaning intensity as a function of the scattering vector, contains the information about the particle shape, size and size distribution [6].

The zeta potential is measured by determining the electrophoretic mobility of the particle or molecule of interest. ELM is the mobility of the particle/molecule under the influence of an applied field relative to the liquid in which it is suspended in [7].

The particle sizing methods and the corresponding equivalent spherical diameters presented in this certification report are summarised in Table 1.

Table 1: Particle sizing methods and the corresponding equivalent spherical diameters measured.

Method	Equivalent spherical diameter
Dynamic light scattering (DLS) — Cumulants method	<ul style="list-style-type: none"> • Harmonic intensity-weighted mean hydrodynamic diameter • Volume-weighted mean diameter
Centrifugal liquid sedimentation (CLS) — line-start method	<ul style="list-style-type: none"> • Intensity-based modal Stokes diameter
Transmission electron microscopy (TEM)/ Scanning electron microscopy (SEM)	<ul style="list-style-type: none"> • Number-based modal diameter
Small angle X-ray scattering (SAXS)	<ul style="list-style-type: none"> • Intensity-weighted mean diameter • Volume-weighted mean diameter

The zeta potential was deduced from the electrophoretic mobility of the silica particles, so the method used was Electrophoretic mobility (ELM). The potentiometric method with a glass electrode is used for pH measurements.

2. Participants

Starting material producer and processing

Chemiewerk Bad Koestritz GmbH (DE) (producer of the raw material);
European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel (BE) (dilution of the starting material and ampouling).

Homogeneity and stability studies

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE (accredited to ISO Guide 34 for production of reference materials, BELAC No 268-TEST).

Characterisation

Participants are listed in alphabetical order. The accreditation body and certificate number are stated for the participants accredited for the measurements.

Agfa-Gevaert NV, Research and Development Materials, Mortsels, BE

Anton-Paar GmbH, Graz, AT

BASF SE, Polymer Physics, GKC/O, G201, Ludwigshafen, DE

Beijing Center for Physical and Chemical Analysis — BCPA, Beijing, CN (accreditation to ISO/IEC 17025, China National Accreditation Service, L0066 2002-10)

Capsulation Pharma AG, Berlin, DE

Federal Institute for Materials Research and Testing, BAM I.3 'Structure Analysis; Polymer Analysis', Berlin, DE

Flemish Institute for Technological Research — VITO, Materials Technology, Mol, BE

Horiba Instruments Inc., Irvine, USA

Industrial Technology Research Institute ITRI, Hsinchu, TW, (CMS/ITRI, accreditation to ISO/IEC 17025, Taiwan Accreditation Foundation, N0688/2000.10.15. NTRC/ITRI, accreditation to ISO/IEC 17025, Taiwan Accreditation Foundation, 1569/2006.8.10.)

Institute for Health and Consumer Protection, JRC, EC, Ispra, IT

Institute for Reference Materials and Measurements — JRC, EC, Geel, BE

Dr. Lerche KG, Berlin, DE

Malvern Instruments Ltd., Malvern, UK

Malvern Instruments Inc., Westborough, USA

Max-Planck-Institute of Colloids and Interfaces, Golm, DE

microParticles GmbH, Berlin, DE

MVA Scientific Consultants, Duluth, US (accredited to ISO/IEC 17025, American Association for Laboratory Accreditation, 2096.01)

National Center for Nanoscience and Technology, Institute of High Energy Physics, CAS Key, Lab for Biological Effects of Nanomaterials and Nanosafety, Beijing, CN

Nanotechnology Characterization Laboratory, Frederick, USA

National Measurement Institute Australia — NMIA, Lindfield, AU

National Physical Laboratory — NPL, Materials Division, Teddington, UK,

National Institute of Standards and Technology — NIST, Gaithersburg, USA

DANNALAB B.V., Enschede, NL

Particle Metrix GmbH, Meerbusch, DE

Philips Research-MiPlaza, Eindhoven, NL

Physikalisch-Technische Bundesanstalt — PTB, Berlin, DE

Rigaku Innovative Technologies Inc., Auburn Hills, USA

RIKILT, Institute of Food Safety, Wageningen, NL (accredited to ISO/IEC 17025ISO, Dutch Accreditation Council, L014)

Sympatec GmbH, Clausthal-Zellerfeld, DE

SIRRIS, Seraing, BE

Technical University Bergakademie Freiberg, Institute MVT/AT, Freiberg, DE

Technical University of Dresden, Institute of Process Engineering and Environmental Technology, Dresden, DE

University of Namur-FUNDP, Nanotoxicology Platform Characterization Group, Namur, BE

University College Dublin, Dublin, IR

3. Processing

3.1. Material selection

The starting material chosen for the production of ERM-FD100 was colloidal silica called Köstrosol 1530 (Chemiewerk Bad Köstritz GmbH, DE). Information about the raw material is summarised in Table 2.

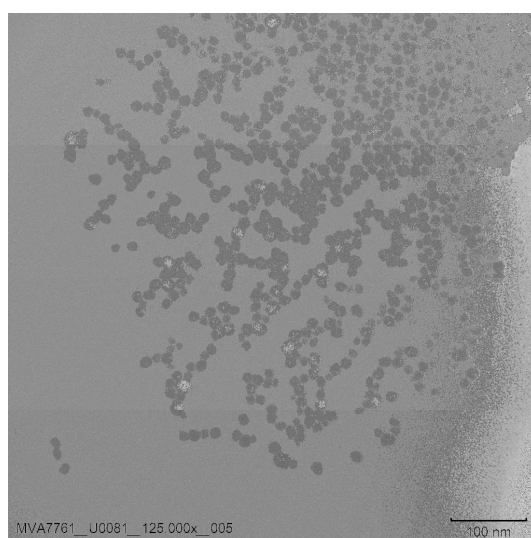
Table 2: Information about the Köstrosol 1530 raw material.

Characterisation data	Köstrosol 1530	Information source
Raw material	Colloidal silica	Manufacturer
Date of production	16.02.2009	Manufacturer
Nominal particle size	15–20 nm	Manufacturer

Dry mass	30.5 % (m/m)	Manufacturer
pH, 25 °C	9.5–10.3	Manufacturer
Viscosity, 25 °C	6 mPa·s	Manufacturer
Particle mass fraction	30.5 % (m/m)	Measurements at JRC, IRMM, RM Unit
Particle morphology	Spherical	TEM measurements commissioned by IRMM
Particle density	2.305 g/cm ³	Literature [20]
Refractive index	1.46	Literature [20]

A TEM image of the Köstrosol 1530 raw material diluted in ultrapure water to 0.2 % (m/m) is shown in Figure 1. A monomodal particle population with a modal diameter of 18 nm was distinguished in the number-weighted particle size distributions (TEM measurements commissioned by IRMM).

Figure 1: TEM micrograph of colloidal silica nanoparticles.



3.2. Processing

The target particle mass fraction for ERM-FD100 was 1 % (m/m); 25 L of a 1 % (m/m) silica suspension were prepared by dilution of 818.88 mL Köstrosol 1530 with 24.18 L deionised water (conductivity 18.2 MΩ·cm¹, Millipore SAS, Molsheim, FR) in a clean plastic tank, mixed by hand-shaking and left standing overnight. The next day, the suspension was transferred from the 25 L plastic tank to a 20 L glass bottle by pumping with a 100 mL dispenser, starting from the top and going to the bottom. The solution up to a filling of about 7 cm was left at the

bottom of the plastic tank and the glass bottle finally contained about 19 L suspension ready for ampouling.

Pre-scored amber glass ampoules of 10 mL were chosen for the processing of ERM-FD100. Before filling, the glass ampoules were opened, rinsed with ultrapure water and dried in an oven. After drying, the ampoules were placed back in carton boxes and the boxes were sealed in plastic bags, ready for use. The bags were opened just prior to processing. The ampoules were filled with approximately 9 mL of colloidal silica solution and flame-sealed.

The suspension in the glass bottle was continuously stirred with a magnetic stirrer throughout the filling process of the ampoules.

A total of 2 064 ampoules of ERM-FD100 were produced. Each ampoule was labelled with an indication of the batch code (ERM-FD100) and an individual identification number.

4. Assessment of homogeneity

A key requirement for any reference material (RM) is the equivalence between the various units. Consequently, ISO Guide 35 requires RM producers to quantify the variation between bottles. This aspect is covered in between-unit homogeneity studies.

Within-unit heterogeneity does not influence the uncertainty of the certified value, but determines the minimum size of a subsample that is representative for the whole unit. Quantification of within-unit heterogeneity is therefore necessary to determine the minimum sample intake.

4.1. Between-bottle homogeneity

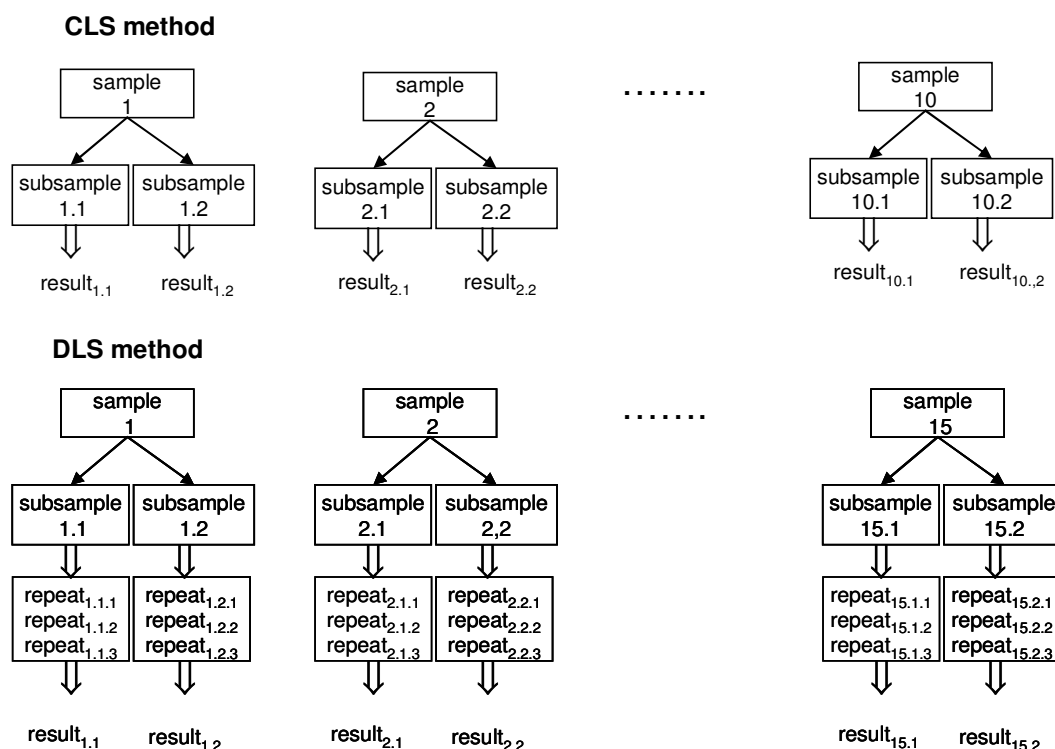
Quantification of between-bottle (=ampoule) heterogeneity can be facilitated by using the analysis of variance (ANOVA), which can separate on the basis of an appropriate measurement set-up the between-bottle variation (s_{bb}) from the within-bottle (=ampoule) variation (s_{wb}). The latter is equivalent to the analytical variation if the individual subsamples are representative for the whole bottle (=ampoule). Evaluation by ANOVA requires that the data for bottle averages follow at least a unimodal distribution and that results for each ampoule follow unimodal distributions with approximately the same standard deviations. In general, the distribution of the ampoule averages can easily be checked. However, too few data are available for each ampoule to make a clear statement of the distribution of individual

results. Therefore, all individual data were checked for unimodality. Minor deviations from unimodality of the individual values do not significantly affect the estimate of between-unit standard deviations. One has to bear in mind that s_{bb} and s_{wb} are estimates of the true standard deviations and therefore subject to random fluctuations. Therefore, the mean square between groups (MSB) can be smaller than the mean squares within groups (MSW), resulting in negative arguments under the square root used for the estimation of the between-bottle variation, whereas the true variation cannot be lower than zero. In this case, u_{bb}^* , the maximum heterogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [8]. u_{bb}^* is comparable to the limit of detection of an analytical method, yielding the maximum heterogeneity that might be undetected by the given study set-up.

During the certification study of ERM-FD100, different methods such as DLS, CLS, SAXS, SEM and TEM were used. Even a method-defined, measurand targeting the same particles is sufficient to confirm homogeneity. If the methods have different precision, then the method with the highest precision should be chosen. Among the tested methods, DLS has the best repeatability; therefore the main assessment was based on DLS. In addition, CLS measurements were performed to confirm the DLS results. Moreover, CLS has the advantage over DLS that it has a much higher sensitivity to detect multimodal distributions than DLS.

Between-bottle (=ampoule) homogeneity was tested on 25 samples, of which 15 samples were tested by DLS performed on a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK) and 10 samples were tested by CLS using a DC20000 Disc CentrifugeTM (CPS Instruments Inc., Stuart, Florida, USA). The samples were taken from the batch using a random stratified sampling scheme, thus ensuring that the complete batch was covered. Two subsamples per ampoule were measured under repeatability conditions by DLS and CLS. Each DLS subsample result was an average of three repeats. The planned and performed measurements are schematically represented in Figure 2. Sample intakes were 1.5 mL for DLS and 0.2 mL for CLS. The measurand of the DLS cumulants method was the intensity-weighted harmonic mean particle diameter and that of the line-start CLS method was the intensity-based modal Stokes particle diameter.

Figure 2: Measurements schemes of the homogeneity study.



A second subset of eight samples covering the whole batch had been taken for the short-term stability STS testing (day 1) during storage and measured by CLS and ELM and for pH. These samples were selected from the whole batch following a random stratified sampling scheme and analysed under repeatability conditions, i.e. in one analytical run (one day). It was observed that both storage time and temperature did not affect the zeta potential of the silica particles and the pH of the suspension. The data from these studies were used to gain additional information on material homogeneity when outliers, bimodal distributions or trends in the filling sequence were found. To do this, storage information was ignored and only the ampoule code was taken into consideration. These data were subjected to the same evaluations as the samples originally used for homogeneity.

Grubbs tests at 99 % confidence interval were performed to detect potentially outlying individual results as well as outlying ampoule averages. Regression analyses were performed to evaluate potential trends in the analytical sequence as well as trends in the filling sequence. It was furthermore checked whether the individual data and ampoule averages follow normal distributions using normal probability plots and whether the individual data are unimodally distributed using histograms.

4.1.1. Descriptive evaluation

The results of these evaluations are shown in Table 3. The measurement results are depicted in Annex A.

Table 3: Descriptive evaluation of the ERM-FD100 homogeneity study results for each method. The DLS measurand is the intensity-weighted harmonic mean particle diameter and the line-start CLS measurand is the intensity-based modal Stokes particle diameter.

Method	Trends		Outliers		Distribution	
	Analytical sequence	Filling sequence	Individual results	Bottle means	Individual results	Bottle means
DLS ⁽¹⁾	no	no	6–technical reason (removed)	none	normal	normal
CLS ⁽¹⁾	yes at 95 % CI	no	4–technical reason (removed)	none	normal	normal
CLS ⁽²⁾	yes at 95 % CI	no	none	none	normal	normal
ELM ⁽²⁾	no	no	none	none	normal	normal
pH ⁽²⁾	no	no	none	none	normal	normal

⁽¹⁾ Data from the homogeneity study were used.

⁽²⁾ Data from the short-term stability (day 1) study were used.

For ERM-FD100 no trends in the filling sequence and in the analytical sequence were found with respect to particle size (DLS), zeta potential (ELM) and pH. For the CLS method, both data from homogeneity and STS studies showed a trend in the analytical sequence at a confidence interval of 95 %, but not at 99 % so no trend correction was applied.

Six outliers were detected in the individual DLS measurement results of the homogeneity study. These outliers corresponded to one of the two replicate measurements of ampoule numbers 172, 688, 1 402, 1 582, 1 857 and 1 930. They could be traced to a measurement position too close to the cell wall because of the plastic cuvettes used for the measurements of ERM-FD100. The Malvern Zetasizer Nano ZS instrument has the possibility to automatically adjust the measurement position in the sample cuvette. As the ERM-FD100 has a small particle diameter of around 20 nm, the samples are right on the edge of being measured at the centre of the cell or near to the wall. Due to the optical quality of the disposable cells being inferior to that of the glass cell, the optimisation protocol occasionally determines the measurement position to be that of the wall. This causes scattering from the

wall of the plastic cuvette giving extra 'noise' and more count rates. Hence, a change of the cell positioning factor of the instrument and a significant contribution of laser flare contained in the detected signal result in increased size and polydispersity index (PI) (www.malvern.com).

Investigation of the outlying values showed that they were all obtained on measurement positions close to the cell wall, whereas all other results were obtained by measurement in the centre of the cell. Therefore, the outliers are technically invalid and were consequently excluded.

Four results of the CLS method (second replicate from ampoule numbers 287, 801, 1 250 and 1 544) were slightly lower than the rest of the results. These were the last performed measurements of the study for the day. No reason can be found to explain the lower results in the particle diameter of ampoules where the first replicate had given results in line with the other results. The fact that these were the last samples to be analysed suggests change of measurement conditions. The data of the STS study by CLS were used to gain additional information on material homogeneity. Using these data, one trend of the analytical sequence could be seen (again at a confidence interval of 95 % but not at 99 %), but no heterogeneity, as the results followed the normal distribution. Therefore the last four measurements by CLS within the homogeneity study were discarded.

Distributions for the individual results were in all cases normal.

From this descriptive evaluation it was concluded that using all accepted data available for ERM-FD100 would result in reliable estimates for the between-bottle heterogeneity.

4.1.2. Quantification of heterogeneity

Results were evaluated using single-factor ANOVA. Standard deviations within bottles (s_{wb}) and between bottles (s_{bb}) as well as u^*_{bb} were calculated. The results of these evaluations are shown in Table 4.

Table 4: Results of the homogeneity study for ERM-FD100.

Method	s_{wb} [%]	s_{bb} [%]	u_{bb}^* [%]
DLS ⁽¹⁾	0.82	n.c.	0.45
CLS ⁽¹⁾	1.91	0.82	1.15
CLS ⁽²⁾	2.54	1.33	1.27
ELM ⁽²⁾	5.99	13.21	2.99
pH ⁽²⁾	0.73	0.58	0.36

⁽¹⁾ Data from the homogeneity study were used.

⁽²⁾ Data from the short-term stability study (day 1) were used.

n.c.= cannot be calculated as $MSB < MSW$.

The occurrence of $MSB < MSW$ (n.c. in Table 4) for the DLS method demonstrates that the material heterogeneity between groups is smaller than that within groups.

The good repeatability of the DLS measurements allowed the setting of very tight limits for potential heterogeneity. The studies demonstrated that the potential between-unit variation in the ERM-FD100 material is 0.45 % for the DLS method. CLS had shown to have worse repeatability in both studies, which did not allow the setting of such tight limits for heterogeneity.

The material is therefore sufficiently homogeneous to serve as reference material for particle size. The huge heterogeneity of the ELM method could come from the fact that the measurements were not performed immediately after opening the ampoule. One of the explanations is that during air exposure the carbon dioxide reacts with water yielding carbonic acid. As a result, changes in particle mobility and zeta potential accordingly are to be expected due to the pH variation of the suspension. Similar scatter of zeta potential results by the ELM method were noticed in the organised interlaboratory comparison (ILC) organised by IRMM and demonstrating a large 'between-bottles' (bottle = ampoule) and 'between- participants' spread [14].

4.2. Within-bottle heterogeneity and minimum sample intake

Within-bottle (= ampoule) heterogeneity is closely correlated to the minimum sample intake. Due to the intrinsic heterogeneity, individual subsamples of a material will not contain the same number and type of particles; hence the mean/modal diameter will change. The

smallest subsample that is representative for the complete ampoule is the minimum sample intake. The larger the intrinsic heterogeneity, the larger the minimum sample intake will be.

Minimum sample intake for the different methods was determined from the results of the method validation studies, the instrument information supplied by the manufacturer and the characterisation study. The sample intake that still yielded results with an accuracy acceptable to be included in the respective studies was taken as minimum sample intake.

The following minimum sample intakes were derived: 200 μL for the CLS analysis and 1 500 μL for the DLS analysis determined from results of the in-house validation study. The minimum sample intake for the line-start CLS method will allow one measurement result.

The minimum sample intake for the SEM should be not less than 50 μL but enough to ensure the measuring of a minimum of 500 particles on the testing grid. The minimum TEM sample intake should be not less than 2.5 μL but enough for the analysis of at least 500 randomly selected particles.

The minimum sample intake for the SAXS method is 20 μL as obtained from the characterisation study.

The instrument information supplied by the manufacturer should be taken into account for the minimum sample intake of the ELM method and pH measurements.

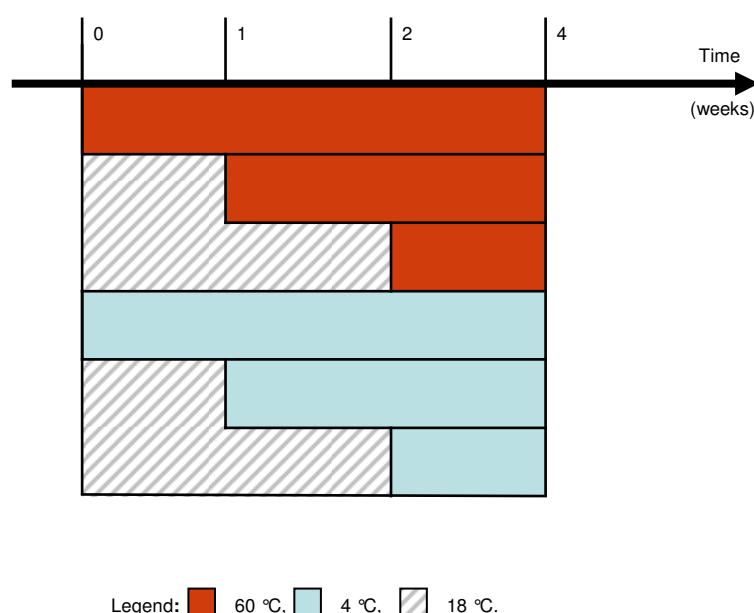
5. Assessment of stability

Stability testing is necessary to establish conditions for transport to the customers as well as conditions for storage. Due to the dilution process, particles might agglomerate or dissolve, even if they were prepared from an initially stable suspension. Time and temperature were regarded as the most relevant influences on the stability of the materials. Therefore, only the influences of these parameters were investigated.

The stability studies were conducted as isochronous stability studies [9]. In this type of study, samples are stored under test conditions for a certain time interval. At the end of the time period, samples are moved to conditions where further degradation is expected to be negligible ('reference condition'), effectively 'freezing' the degradation status of the materials. This set-up allows analysis of materials of various exposure times under repeatability conditions, thus greatly improving the sensitivity of the study to detect degradation.

A subset of 14 ampoules covering the whole batch had been taken for the STS testing. The samples were selected following a random stratified sampling scheme and were analysed under repeatability conditions. Samples were stored for 0, 1, 2 and 4 weeks at 4 °C and 60 °C, respectively. No tests were done at temperatures below 4 °C as previous experience with similar materials had shown that irreversible sedimentation occurs when the sample is frozen. Storage at 18 °C was defined as reference condition for the short-term stability study. The storage design for the isochronous STS study is shown in Figure 3.

Figure 3: Storage scheme for the isochronous measurements – STS study.



Two ampoules were analysed for each time/temperature combination. At the end of the study, measurements on two independent subsamples were performed on each ampoule by CLS, DLS, ELM and pH. For ELM and DLS, each subsample result consisted of three repetitive measurements (Figure 2). The two aliquots of DLS and ELM subsamples were combined together with the remaining suspension of each ampoule. The sample was then split into two aliquots. The pH was measured in three repeats.

In total four independent results per time/temperature combination and per method were obtained (2 ampoules x 2 aliquots).

A subset of 32 ampoules covering the whole batch had been taken for the long term stability (LTS) testing. Samples were stored for 0, 4, 8 and 12 months at 18 °C, using 4 °C as a reference temperature. Eight ampoules were stored for each time. At the end of the study,

measurements were performed on these 32 ampoules. From each opened ampoule, six aliquots were taken (two for CLS, two for ELM and two for DLS) and measured in parallel in the same way as in the short-term study. The ELM and DLS aliquots were again measured in three repetitions.

In total, 16 results per time and per method were obtained (8 ampoules x 2 replicates).

The studies were evaluated individually for each temperature. Results were screened for outliers using a Grubbs test, but only outliers that were dubious on technical grounds were excluded. Linear regressions were performed and the slopes were tested for significance at a 95 % confidence interval. The results of these evaluations are summarised in Tables 5 and 6, whereas graphical representations of the studies are given in Annex B.

The measurands for DLS and line-start CLS are the intensity-weighted harmonic mean particle diameter and the intensity-based modal Stokes particle diameter, respectively, and the zeta potential for the ELM method.

Table 5: Results of the evaluation of the four-week STS.

Temperature	4 °C		60 °C	
Method	Outliers 95 % conf.	Slope	Outliers 95 % conf.	Slope
DLS	1 (retained)	no	none	yes ⁽²⁾
CLS	none	no	none	no
ELM	1 (retained)	no	none	no
pH	none	yes ⁽¹⁾	1 (retained)	no

⁽¹⁾ Slope significant at 95 % confidence interval

⁽²⁾ Slope significant at 95 % and 99 % confidence interval

Table 6: Results of the evaluation of the one-year LTS.

Temperature	18 °C	
Method	Outliers	Slope
DLS	1 (retained)	not significant
CLS	1 (retained)	not significant
ELM	1 (retained)	not significant

Some outlying individual results were found for the DLS and ELM methods during the 4 °C STS but the results were retained, as no technical reason was found. The slope of the DLS STS at 60 °C was found to be significant on a 99 % level.

Outlying results were also found for the DLS, CLS and EM methods in the LTS study at 18 °C. However, removal of these results did not affect the result on the significance of the slopes. As no technical reason for the outliers could be identified and since the retention leads to a conservative estimate for the uncertainty of degradation, the results were retained.

Uncertainties of stability during dispatch and storage were estimated as described in [10] for each method. For this approach, the uncertainty of the linear regression line with a slope of zero is calculated. The uncertainty contribution is then estimated as a chosen shelf life multiplied with the uncertainty of the regression lines. The following uncertainties were estimated:

- u_{sts} , the uncertainty of the particle size during dispatch. This was estimated from the 60 °C studies for a time period of one week. The uncertainty therefore describes the possible change during a dispatch at 60 °C lasting for one week. For DLS, the extent of degradation was added as a rectangular distribution accounting for the slope significance (Table 7);
- u_{lts} , the stability during storage. This uncertainty contribution was estimated from the 18 °C studies or from the combined studies for a shelf life of 24 months. The uncertainty contribution therefore describes the possible degradation for 24 months at 18 °C.

The results of these evaluations are summarised in Table 7.

Table 7: Results of the evaluation of the relative uncertainties of stability for ERM-FD100. u_{sts} was estimated for a period of one week storage at 60 °C. u_{lts} was estimated for a storage of 24 months at 18 °C from the one year/18 °C study.

Method	u_{sts} 60 °C, 1 week [%]	u_{lts} 18 °C, 24 months [%]
DLS	0.33	1.09
CLS	0.45	2.46
ELM	2.58	21.00
pH	0.14	not measured

As can be seen in Table 7, u_{sts} is negligible in all cases compared to the uncertainty of long-term stability. The ERM-FD100 material can be therefore dispatched under ambient conditions.

Uncertainties of stability during long-term storage are about 1.1 % for the DLS method and 2.5 % for the CLS method. These uncertainties are taken up in the final uncertainty budget of the certified values. The uncertainty of the ELM method is 21.0 % due to the significantly worse repeatability and might be reduced in future, based on the results of the post-certification stability monitoring.

6. Characterisation

6.1. Selection of participants

Participants in the characterisation studies were selected based on criteria that comprised both technical aspects as well as aspects regarding quality management.

Participants for the characterisation had demonstrated their competence and proficiency to perform particle size measurements on aqueous suspensions of silica nanoparticles in the 10 nm to 100 nm size range during an ILC, organised by IRMM [14] or other ILCs of their choice.

Fulfilment of the quality management requirements ensured that the technical standard was maintained from the time of demonstration in the ILC to the actual measurement. Only few participating laboratories are accredited to ISO/IEC 17025. Where measurements are covered by the scope of accreditation, the accreditation number is stated in the list of participants (Section 2).

The scatter of the zeta potential results by the ELM determination in the ILC organised by IRMM was too large to allow a positive demonstration of proficiency. However, since the scatter was most likely caused by the properties of the test material (TS-2009/1), it was decided to open the ELM characterisation study to all interested laboratories. Consequently, the zeta potential value obtained from the characterisation study was given as an indicative value rather than a certified value.

6.2. Set-up

The aim of the study was the characterisation of the colloidal silica candidate reference material via an interlaboratory study with subsequent technical evaluation. The basis of the certification approach was the randomisation of the (unknown) laboratory biases. Randomisation is only successful if,

- (1) the measurements within one laboratory are performed under reproducibility conditions (to minimise the bias of the laboratory's result), and
- (2) results from different laboratories are indeed independent.

In this respect, the participating laboratories were asked to strictly follow the test protocol distributed together with the samples. This protocol included tests on a quality control material (QCM), and the requirement to register and report all the requested test details into a specially prepared test report form. Both documents, the test protocol and the test report form, were prepared for each of the five methods used in this characterisation study. The instruments and the methods used are summarised in Annex C. The information in this annex is presented as reported by the participants.

6.2.1. DLS

The characterisation of the colloidal silica candidate CRM by the DLS method was performed in terms of the intensity-weighted harmonic mean particle diameter of particle size distribution, in general accordance with the standards ISO 22412 [15] and ISO 13321 [16]. This certification report includes the results from the cumulants and not from the frequency analysis method. Information about some physical properties of the silica material was given to the participants (e.g., a particle density of 2.3 g/cm³ and refractive index of 1.46 [20]).

A QCM, colloidal silica with a nominal particle mass fraction of 0.75 % (m/m) and a particle size diameter in the range of 20 nm to 50 nm, the same material as used for the ILC [14], was sent together with the samples. The QCM and the test sample material consisted of approximately 9 mL of the silica suspension contained in 10 mL pre-scored amber glass ampoules.

6.2.2. CLS

The measurand of the CLS method was the intensity-based modal Stokes particle diameter corresponding to the main mode in the particle size distribution of the colloidal silica candidate reference material, in general accordance with the standard test methods ISO 13318-1:2001 [17] and ISO 13318-2:2007 [18]. This includes both the line-start method and homogeneous techniques, implemented in disc centrifuges or cuvette centrifuges.

The QCM was the same as the one used for the DLS.

Participants were instructed to use a particle density of 2.3 g/cm^3 for the evaluation of their measurements.

6.2.3. EM (TEM/SEM)

The characterisation of the colloidal silica candidate CRM by EM in terms of the modal diameter of the main mode in their area equivalent particle size distribution was based on the analysis of electron microscopy images in general accordance with the standard static image analysis method ISO 13322-1:2004 [19]. This includes both scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques.

The same QCM as for the DLS was also used in this case.

6.2.4. SAXS

The colloidal silica candidate CRM was also characterised in terms of particle size using Small Angle X-ray Scattering (SAXS).

A QCM (RM 8012, nominal particle size 30 nm, NIST, Gaithersburg, US) was sent with the sample test material. It consisted of a suspension of colloidal Au with a nominal particle mass fraction of 48 % and a particle diameter in the range of 20 nm to 40 nm. This QCM consists of approximately 5 mL Au suspension in a 5 mL pre-scored amber glass ampoule.

6.2.5. ELM and pH

The colloidal silica candidate CRM was characterised in terms of pH and zeta potential, the latter via electrophoretic mobility measurements. Carbon dioxide from air reacts with water and as a result, changes in zeta potential are to be expected after opening an ampoule due

to the changing of pH. Therefore, it was explicitly mentioned that immediately after opening an ampoule, two independent aliquots had to be prepared, protected from air, and tests had to be performed as soon as possible.

The QCM consisted of polystyrene latex microspheres dispersed in an aqueous buffer (pH 9.2) and packed in a 10 mL syringe (Zeta Potential Transfer Standard, Malvern Instruments Ltd, UK).

6.2.6. Measurement protocol

The participants received three ampoules of ERM-FD100 and one QCM ampoule. In total, two independent results (= from independent subsamples or 'aliquots') per ampoule (ERM-FD100) had to be delivered together with two results for the QCM.

On the first day, the measurements on two aliquots of ERM-FD100 and two aliquots of the quality control material had to be performed. On days 2 and 3, only two aliquots of ERM-FD100 (no quality control sample) had to be measured. Each aliquot had to be measured in triplicate (= three instrument readings). This measurement schedule, which had to be strictly followed by the participants, is shown in Table 8. It was requested to measure the suspensions in the ampoules as received and not to do any sample pre-treatment prior to the measurements (i.e. without filtration, dilution, centrifugation, sonication).

Table 8: Overview of the measurements to be performed on the three different days.

	Day 1	Day 2	Day 3
1	QCM aliquot 1	ERM-FD100 aliquot 1 ampoule 2	ERM-FD100 aliquot 1 ampoule 3
2	ERM-FD100, aliquot 1 ampoule 1	ERM-FD100 aliquot 2 ampoule 2	ERM-FD100 aliquot 2 ampoule 3
3	ERM-FD100 aliquot 2 ampoule 1	—	—
4	QCM aliquot 2	—	—

The participating laboratories were also requested to give estimations of the expanded uncertainties of the mean result for each ampoule. No approach for the estimation was

prescribed, i.e. top-down and bottom-up approaches were regarded as equally valid procedures.

6.3. Technical evaluation

Thirty-four laboratories took part in the characterisation study, most of them offering several different methods (see Annex C) and measurement results. The geographical distribution of the participants was as follows: 6 from the United States, 3 from Asia, 24 from Europe and 1 from Australia.

Before starting the technical evaluation, it was checked if the results of the QCMs provided for each method agreed with the assigned values in the NIST certificate for the SAXS method, the Malvern certificate for the ELM method and the mean value of the data from the ILC study. There were no results that differed significantly; therefore none of them were rejected.

6.3.1. DLS results

Seventeen participants submitted 19 independent data sets for the DLS cumulants method. Thirteen laboratories submitted results of both intensity and volume-weighted particle diameter. Laboratories 5, 6, 10, 12 and 20 only submitted intensity-weighted data. Laboratories 17 and 18 reported two datasets for the cumulants analysis method obtained from two independent instruments.

Four data sets were reported for the DLS frequency analysis method. The frequency data were significantly higher than the cumulants method data and therefore they could not be evaluated together. Since the number of available data sets for the DLS frequency analysis method was too low for statistical evaluation, it was decided not to include these results in the current certification report. The frequency analysis DLS method results are presented in a graph in Annex D.

No effect of the scattering angle on the DLS cumulants results was noticed while analysing the data. No participant reported specific problems with measuring the samples. All these data sets are based on results of undiluted samples.

6.3.2. CLS results

Ten participants submitted 10 independent data sets of which six were obtained by line-start disc centrifugal liquid sedimentation (also called differential centrifugal sedimentation), and four by homogeneous centrifugal liquid sedimentation (also called analytical ultracentrifugation or photocentrifuge). From the four homogeneous CLS data sets only three participants reported an intensity-based modal particle diameter.

Data from the homogeneous CLS method differed significantly from the line-start method. Two laboratories reported results from the homogeneous CLS method of around 15 nm for the intensity-based particle diameter and a third laboratory reported around 25 nm, i.e. with a difference of 10 nm. Therefore, data could not be pooled together. Only the six data sets of the CLS line-start method were further evaluated. The other results are listed in Annex D.

6.3.3. EM (TEM/SEM) results

Eight participants submitted 11 independent data sets of which eight were obtained by TEM and three by SEM. Laboratory 3, 11 and 15 delivered results both for TEM and SEM. Each laboratory performed the analysis on at least 500 randomly selected and counted particles. The following observations were made.

- TEM laboratory 16 stated that all as-received material samples were diluted to achieve a target mass fraction of 0.075 in order to produce a good coverage of particles on the grids. TEM laboratory 20 diluted 1:50 (v/v) the test material ERM-FD100 before measuring.
- Laboratory 11 stated volume dilution of 1:10 and additional filtration with 0.1 µm filter size for both TEM and SEM measurements.
- SEM laboratory 15 reported a dilution of 1:50 (v/v).

All dilutions were performed with ultrapure water. Each laboratory performed the analysis on at least 500 randomly selected and counted particles.

The SEM and TEM results were pooled as the data fit into one distribution.

6.3.4. SAXS results

Five participants submitted five independent data sets. One laboratory did not report the volume-weighted particle size distribution. The participants reported the intensity- and volume-weighted Guinier radius for intensity and volume-weighted results. The SAXS particle diameter d_{SAXS} was obtained via the following equation 1 [6]:

$$d_{\text{SAXS}} = 2\sqrt{\frac{5}{3}} \cdot r_{\text{Guinier}} \quad \text{Eq. 1}$$

where r_{Guinier} is the reported Guinier radius.

6.3.5. ELM and pH results

Nine participants submitted nine independent data sets for the ELM method and pH. No effect of the scattering angle used for the measurements on the zeta potential results was noticed.

The instruments used for all the methods are summarised in Annex C. The results of these studies are depicted in Annex D. The graphs in Annex D show expanded uncertainties, not standard deviations. Results with a low standard deviation may well have a large uncertainty. Laboratories 11 and 12 did not report any uncertainties for the DLS results. Their uncertainty was calculated and given as the confidence interval (CI) of a mean through the following equation 2:

$$CI = \frac{s}{\sqrt{n}} \cdot t \quad \text{Eq. 2}$$

where s is the standard deviation of the results, n is the number of replicates from which the average was made, t is the t-factor for the 95 % probability and degrees of freedom ($n-1$).

6.4. Statistical evaluation

The data sets accepted on technical grounds were tested using the Grubbs test for outlying means and using the Cochran test for outlying standard deviations (both at a 99 % confidence interval) as well as for normality of data set means using kurtosis/ skewness tests and normal probability plots. Standard deviation within (s_{within}) and between (s_{between}) laboratories were calculated using one-way ANOVA. The results of these evaluations are shown in Table 9.

Table 9: Statistical evaluation of the technically accepted data sets.

Measurand/ method	<i>p</i>	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Average [nm]	<i>s</i> [nm]	<i>s</i> _{between} [nm]	<i>s</i> _{within} [nm]
Intensity-weighted DLS cumulants	19	Lab 24	Labs 12, 15	no	19.00	0.61	0.60	0.24
Volume-weighted DLS cumulants	14	none	none	no	16.86	1.14	0.60	0.24
Intensity-based modal CLS line-start	6	none	Lab 9	yes	20.13	0.68	0.63	0.54
Number based, modal EM (TEM/SEM)	11	none	Lab 11a	yes	19.37	1.99	1.95	0.88
Intensity-weighted SAXS	5	none	Labs 29, 30	yes	21.79	0.34	0.33	0.22
Volume-weighted SAXS	4	none	Lab 30, 31	yes	20.37	0.96	0.95	0.26
Zeta potential, ELM	9	none	none	yes	−43.02 [mV]	5.12 [mV]	4.70 [mV]	4.98 [mV]
pH	9	none	Labs 16, 19, 27, 35	yes	9.66	0.31	0.29	0.18

p: number of accepted data sets of results; *s*: standard deviation of the data set means; averages and standard deviations expressed in nm for CLS, DLS, EM (SEM/TEM), SAXS, mV for the ELM and unit one for the pH.

Outliers of variance show that repeatability varies from laboratory to laboratory. The heterogeneity of variance also prevents pooling all individual results, so the evaluation was based on the mean of laboratory means. Apart from this, outlying variances are not a reason for exclusion of data.

None of the methods shows outliers based on mean values or deviation from normal distribution, except DLS.

The distribution of the intensity-weighted harmonic mean particle diameter (DLS cumulants method) results was unimodal, non-normally distributed with one outlier detected during the statistical evaluation. However, the range of results (18.28 nm to 20.75 nm) is small enough to evaluate them and to assign a single certified value.

The standard deviation for the DLS volume-weighted results is much larger than the intensity-weighted results for the same method as well as the distribution of the results found to be bimodal. That is why the values for the volume-weighted DLS method were not certified, but given as additional information on the certificate.

7. Value assignment

For ERM-FD100, certified, indicative and additional material information values have been assigned.

Certified values are values that fulfil the highest standards of accuracy. Procedures at IRMM require generally pooling of not less than six independent data sets to assign certified values. Full uncertainty budgets in accordance with the ISO/IEC Guide 98-3 [11] must be established.

Indicative values are values where either the uncertainty is deemed too large or where too few independent data sets are available to allow certification. Uncertainties are evaluated according to the same rules as for the certified values.

Additional material information refers to values that have been obtained in the course of the study. For example, results reported from only one or two laboratories or in cases where the individual measurement uncertainty is unacceptably high, would fall into this category.

7.1. Certified values

The unweighted means of the means of the accepted data sets as shown in Table 10 were used as assigned values for all measurands.

The certified uncertainty consists of uncertainties related to characterisation (u_{char}), between-bottle heterogeneity (u_{bb}), potential degradation during long-term storage (u_{its}) and during transport to the customer (u_{sts}) [9].

- u_{char} was estimated as the standard error of the mean of laboratory means, i.e. s/\sqrt{p} with s and p taken from Table 9.
- u_{bb} was estimated as the larger value of the standard deviation between units (s_{bb}) or the maximum heterogeneity potentially hidden by method repeatability (u_{bb}^*). The values are taken from Table 4. The DLS method u_{bb} value of 0.45 % was also taken for the SAXS and EM (TEM/SEM) particle size determining techniques. The higher of the s_{bb} and u_{bb}^* values was taken to estimate the u_{bb} corresponding to CLS, ELM and pH methods.
- u_{sts} was estimated from short-term stability tests and was taken from Table 7 (one week at 60 °C).
- u_{its} was estimated from long-term stability tests and was taken from Table 7 (24 months of storage at 18 °C).

The DLS method u_{sts} and u_{its} values were also taken for the SAXS and EM (TEM/SEM) particle size determining techniques.

These uncertainties were regarded as uncorrelated and therefore they were combined quadratically to estimate the uncertainty of the certified value (u_{CRM}) as shown in equation 3:

$$u_{\text{CRM}} = \sqrt{u_{\text{char}}^2 + u_{\text{bb}}^2 + u_{\text{its}}^2 + u_{\text{sts}}^2} \quad \text{Eq. 3}$$

The various uncertainty contributions and the expanded combined uncertainty are shown in Table 10.

Table 10: Uncertainty budget (relative uncertainties) and certified values.

Equivalent spherical diameter/method	u_{char} [%]	u_{bb} [%]	u_{its} [%]	u_{sts} [%]	u_{CRM} [%]	Certified value [nm]	U_{CRM} ($k = 2$) [nm]
Intensity-weighted harmonic mean, cumulants' DLS	0.73	0.45	1.09	0.33	1.43	19.00	0.55
Intensity-based modal Stokes, line-start CLS	1.38	1.33	2.46	0.45	3.15	20.13	1.27

Number-based modal, EM (TEM/SEM)	3.09	0.45	1.09	0.33	3.33	19.37	1.29
Intensity-weighted mean, SAXS	0.70	0.45	1.09	0.33	1.41	21.79	0.62

u_{Its} is the dominant uncertainty source for the DLS, CLS and SAXS methods. u_{Its} has 62 degrees of freedom, satisfying a coverage factor of 2. For EM (TEM/SEM), u_{char} dominates the uncertainty budget. In this case u_{char} has 10 degrees of freedom, justifying also a coverage factor $k = 2$.

A graphical depiction of all assigned values, together with averages and standard deviations and submitted uncertainties of the individual laboratories is shown in Annex D.

7.2. Indicative values

Indicative values were assigned for the following measurands and methods:

- volume-weighted mean particle size measured by SAXS. Although only four out of the five laboratories had reported volume-weighted particle diameter, the results were regarded as sufficiently trustworthy to assign indicative values.
- zeta potential by the ELM. As the results for the ELM method of the laboratories participating in the feasibility study were not unambiguous, the ILC results of the participating laboratories could not be used to demonstrate their competence. Therefore, a priori we could not certify these values.

The uncertainty budgets were set up as for the certified values and are listed together with the assigned values in Table 11.

Table 11: Uncertainty budget and indicative values.

Equivalent spherical diameter/method	u_{char} [%]	u_{bb} [%]	u_{Its} [%]	u_{sts} [%]	u_{CRM} [%]	Indicative value [nm]	U_{CRM} ($k = 2.8$) [nm]
Volume-weighted mean, SAXS	2.36	0.45	1.09	0.33	2.66	20.37	1.52

Measurand/method	u_{char} [%]	u_{bb} [%]	u_{its} [%]	u_{sts} [%]	u_{CRM} [%]	Indicative value [mV]	U_{CRM} ($k = 2$) [mV]
Zeta potential, ELM	3.97	13.21	21.00	2.58	25.26	−43.02	21.73

For the SAXS method, u_{char} dominates the uncertainty budget. In this case u_{char} has 4 degrees of freedom, justifying a coverage factor $k = 2.8$.

7.3. Additional material information

Additional values are given for the following measurands and methods:

- volume-weighted harmonic mean particle diameter measured by DLS. Although only 14 out of the 19 laboratories had reported volume-weighted particle diameter, the results were regarded as sufficiently trustworthy to assign an informative value;
- pH measurement results. As the results of the pH measurements of the laboratories participating in the feasibility study were not so unambiguous, the ILC results of the participation laboratories could not demonstrate their competence.

8. Metrological traceability

Measurement results with the same established metrological traceability can be compared independently wherever and whenever they are obtained.

The harmonic intensity or volume-weighted arithmetic average particle diameter of the DLS method is operationally defined by ISO 22412:2008 [15] and ISO 13321:1996 [16]. As DLS is an absolute method and does not require calibration, the measurement results expressed in nanometre [nm], are traceable to SI via the monochromatic wavelength of the laser light (Annex C).

The measurand of the line-start CLS method is the intensity-based modal Stokes particle diameter and is operationally defined by ISO 13318-1:2001 [17] and 13318-2:2007 [18] and assuming a particle density of 2.305 g/cm³ [20]. The quantity value is expressed in nanometre [nm]. The results are SI traceable, as a calibrant is used with an SI traceable certified value. The types of calibrants used in each laboratory are shown in Annex C.

The particle diameter obtained with the EM (SEM/TREM) method is SI traceable via the calibrant used and presented in Annex C.

The particle diameter established via SAXS is SI traceable via the wavelength of the X-rays used in the instruments for measurement.

All the methods and instruments used for the ERM-FD100 characterisation and certification are presented in Annex C.

9. Commutability

CRMs must exhibit the same analytical behaviour for given methods as a normal laboratory sample.

The ERM-FD100 is derived from an industrial material and was only diluted. The measurands are method-defined and standard methods are used. Therefore, commutability is not an issue, as the values are valid for the specific methods only.

10. Summary of results

A summary of the final values with corresponding rounding is presented in Tables 12, 13 and 14.

Table 12: Certified values.

Measurand/method	Certified value $\pm U_{\text{CRM}}$ [nm]
Intensity-weighted harmonic mean particle diameter as determined by DLS cumulants method (ISO 22412:2008)	19.0 \pm 0.6
Intensity-based modal Stokes particle diameter as determined by CLS line-start method (ISO 13318-1:2001)	20.1 \pm 1.3
Number-based modal particle diameter as determined by EM (TEM/SEM) (ISO 13322-1:2004)	19.4 \pm 1.3
Intensity-weighted mean particle diameter as determined by SAXS	21.8 \pm 0.7

Table 13: Indicative values.

Measurand/method	Indicative value $\pm U_{\text{CRM}}$ [nm]
Volume-weighted mean particle diameter as determined by SAXS	20.4 ± 1.6

Measurand/method	Indicative value $\pm U_{\text{CRM}}$ [mV]
Zeta potential as determined by ELM	-43.0 ± 21.8

Table 14: Additional material information.

Measurand/method	Additional value [nm]
Volume-weighted mean particle diameter as determined by DLS method (ISO 22412:2008)	16.9

Measurand/method	Additional value
pH as determined by potentiometric method with a glass electrode	9.7

11. Instructions for use

11.1. Storage conditions

The material shall be stored at 18 ± 5 °C. Ampoules should not be allowed to freeze, as this will irreversibly compromise the integrity of the material.

11.2. Safety and protection of the environment

The usual laboratory safety measures apply.

This material should be handled with care. Nanoparticles may have an impact on environment and human health. Any spillage of the suspension should be handled according to the usual laboratory safety precautions.

11.3. Handling of the material

Before opening the ampoule, it should be gently inverted several times to ensure the homogeneity of the suspension and re-suspension of any settled particles. If some suspension is still present in the upper portion of the ampoule (the nipple), it can be removed by gently flicking the nipple with the forefinger while tilting the ampoule. The ampoule is pre-scored and can be opened by applying moderate pressure with one's thumb to snap off the nipple.

DLS method: The contents of an ampoule should be used at the same day as opened without any dilution when used for the DLS method. The use of quartz cuvettes is recommended for the measurement. Manual adjustment of the measurement position to the middle of the cell may be needed before applying the DLS method. A refractive index of 1.46 for the silica was used.

CLS method: A density of 2.3 g/cm^3 and a refractive index of 1.46 were taken for the evaluation of the results. These figures should be used in laboratory calculations and instrument procedure set-up.

EM method (TEM/SEM): A drop of the sample should be put on a holder/grid; after drying at least 500 particles should be measured. If necessary the sample can be diluted with distilled water.

SAXS method: Samples to be measured as received.

Zeta potential and pH should be measured immediately after opening (no storage in air).

11.4. Use of the certified values

The material is intended to be used for method validation and for demonstration of laboratory proficiency.

Comparing an analytical result with the certified value

A result is unbiased if the combined uncertainty of measurement and certified value covers the difference between the certified value and the measurement result (see also ERM Application Note 1 [13]).

Use in quality control charts

The materials can be used for quality control charts. Different CRM units will give the same result as heterogeneity was included in the uncertainties of the certified values.

Use as a calibrant

This material can be used as calibrant for methods for which it has certified values. The uncertainty of the certified value shall be taken into account in the final estimation of measurement uncertainty.

References

- [1] ISO/TS 27687, Nanotechnologies — Terminology and definitions for nano-objects — Nanoparticle, nanofibres and nanoplates (2008).
- [2] Emons H., 'The "RM family" — Identification of all its members', *Accreditation Quality Assurance* 10: 690–691 (2006).
- [3] Couteau O., Charoud-Got J. and Roebben G., 'Processing and value assignment for IRMM-304', Product information sheet, (<http://irmm.jrc.ec.europa.eu/catalogue>) (2008).
- [4] Xu R., Particle characterization: light scattering methods, Kluwer Academic Publishers, Dordrecht (2001).
- [5] Goldstein J., Newbury D., Joy D., Lyman C., Echlin P., Lifshin E., Sawyer L. and Michael J., *Scanning Electron Microscopy and X-Ray Microanalysis*, Springer Science — Business Media, LLC (2003).
- [6] Glatter O. and Kratky O., *Small angle X-ray scattering*: 80,155 (1982).
- [7] Hunter R., *Zeta potential in colloid science — principles and applications*, Academic Press (1981).
- [8] Linsinger T.P.J., Pauwels J., van der Veen A.M.H., Schimmel H. and Lamberty A., 'Homogeneity and stability of reference materials', *Accreditation and Quality Assurance* 6: 20–25 (2001).
- [9] Lamberty A., Schimmel H. and Pauwels J., 'The study of the stability of reference materials by isochronous measurements, Fresenius', *Fresenius' Journal of Analytical Chemistry* 360: 359–361 (1998).
- [10] Linsinger T.P.J., Pauwels J., Lamberty A., Schimmel H., van der Veen A.M.H. and Siekmann L., 'Estimating the uncertainty of stability for matrix CRMs', *Fresenius' Journal of Analytical Chemistry* 370: 183–188 (2001).
- [11] ISO/IEC Guide 98-3, *Uncertainty of measurement — Part 3: Guide to the expression of uncertainty in measurement*, Geneva (1995).
- [12] Pauwels J., van der Veen A., Lamberty A. and Schimmel H.G., 'Evaluation of uncertainty of reference materials', *Accreditation and Quality Assurance* 5: 90–95 (2000).
- [13] Linsinger T.P.J., ERM Application Note 1, (www.erm-crm.org) (2005).
- [14] Lamberty A., Franks K., Braun A., Kestens V., Roebben G. and Linsinger T.P.J., *Interlaboratory comparison of methods for the measurement of particle size, effective particle density and zeta potential of silica nanoparticles in an aqueous solution*, Report-10-003, Joint Research Centre, Institute for Reference Materials and Measurements (2010).

- [15] ISO 22412, Particle size analysis — Dynamic light scattering (DLS) (2008).
- [16] ISO 13321, Particle size analysis — Photon correlation spectroscopy (1996).
- [17] ISO 13318-1, Determination of particle size distribution by centrifugal liquid sedimentation methods — Part 1: General principles and guidelines (2001).
- [18] ISO 13318-2, Determination of particle size distribution by centrifugal liquid sedimentation — Part 2: Photocentrifuge method (2007).
- [19] ISO 13322-1, Particle size analysis — Image analysis methods — Part 1: Static image analysis methods (2004).
- [20] Laven J. and Stein H.N., Journal of Colloid and Interface Science, 238: 8–15 (2001).

Acknowledgements

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Annexes

Annex A: Results of the homogeneity studies

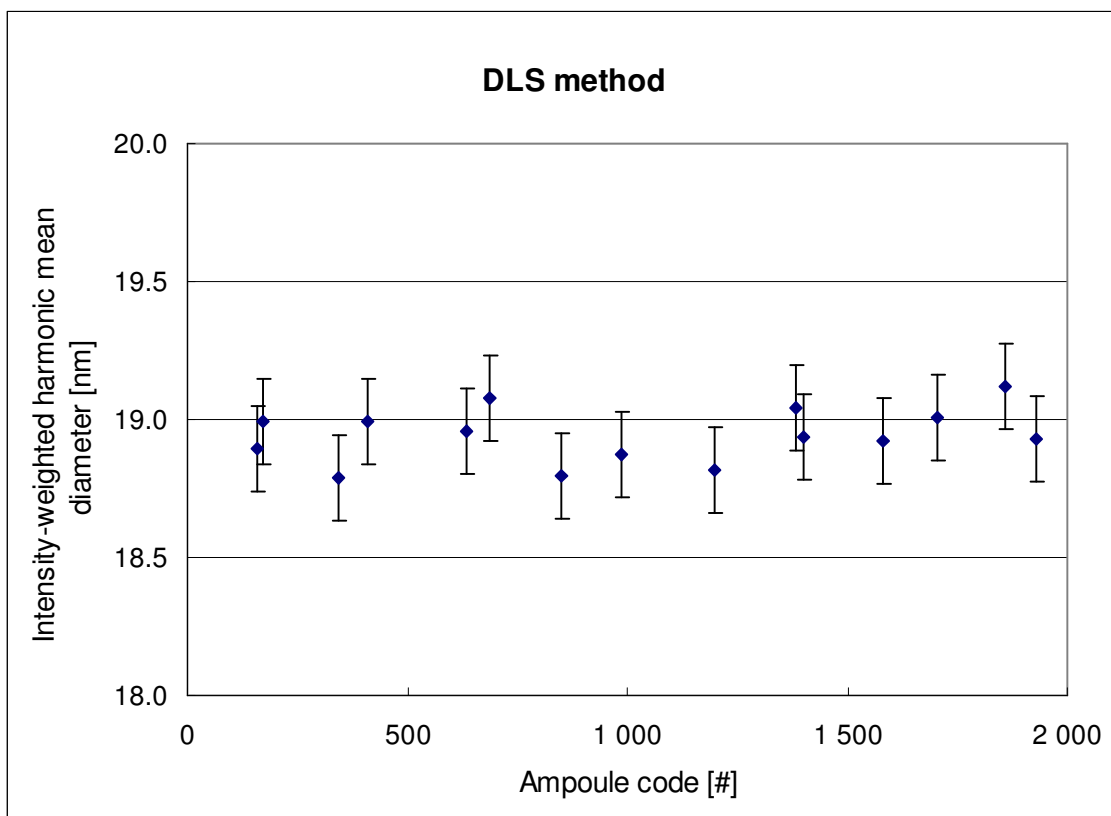
Annex B: Results of the stability studies

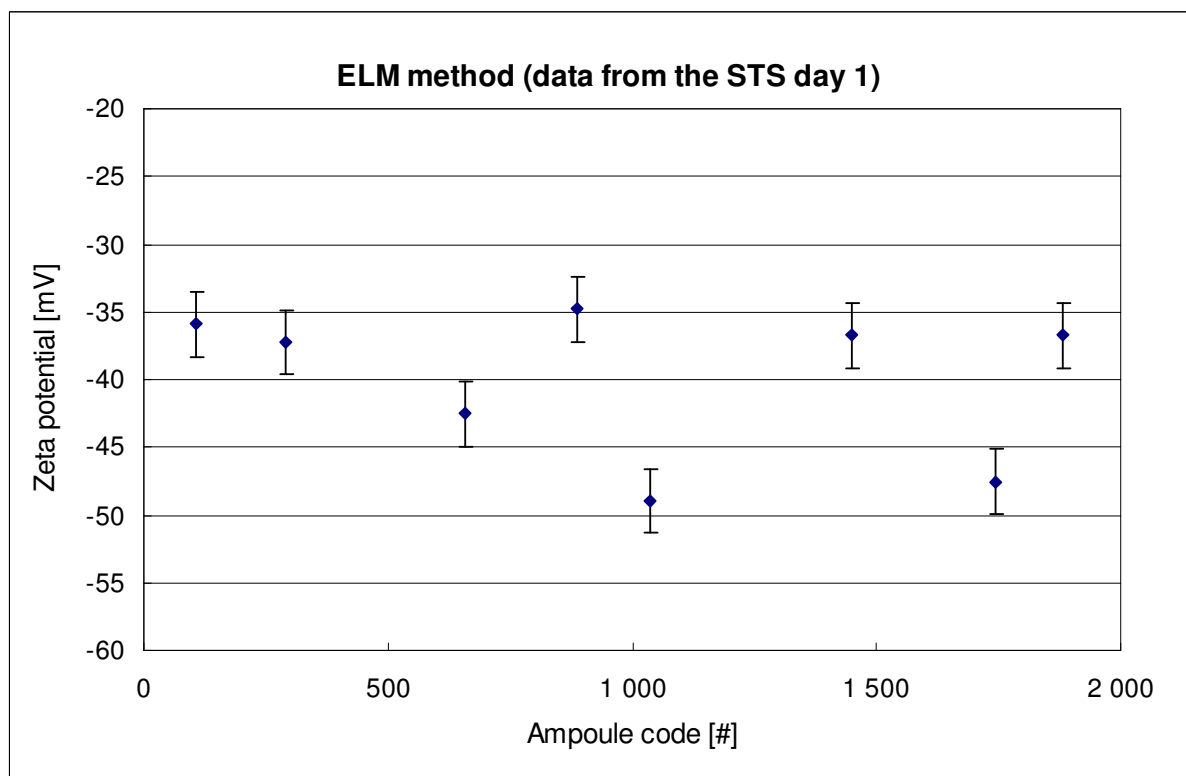
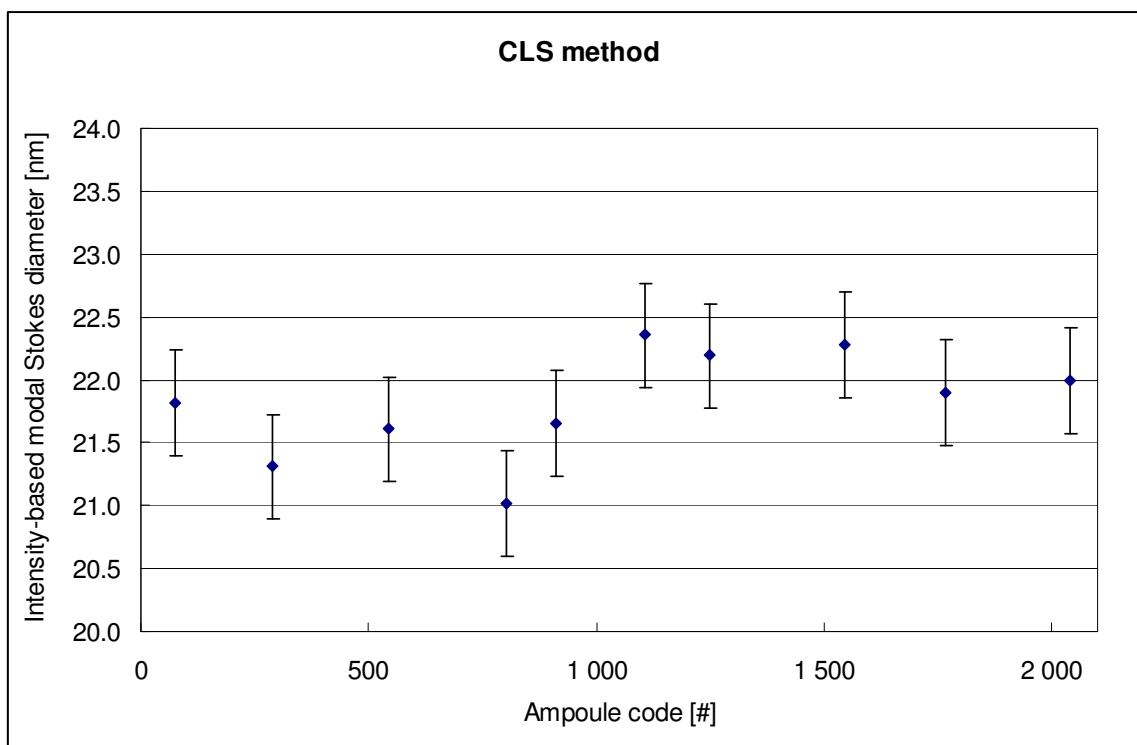
Annex C: Instruments and methods used for the characterisation

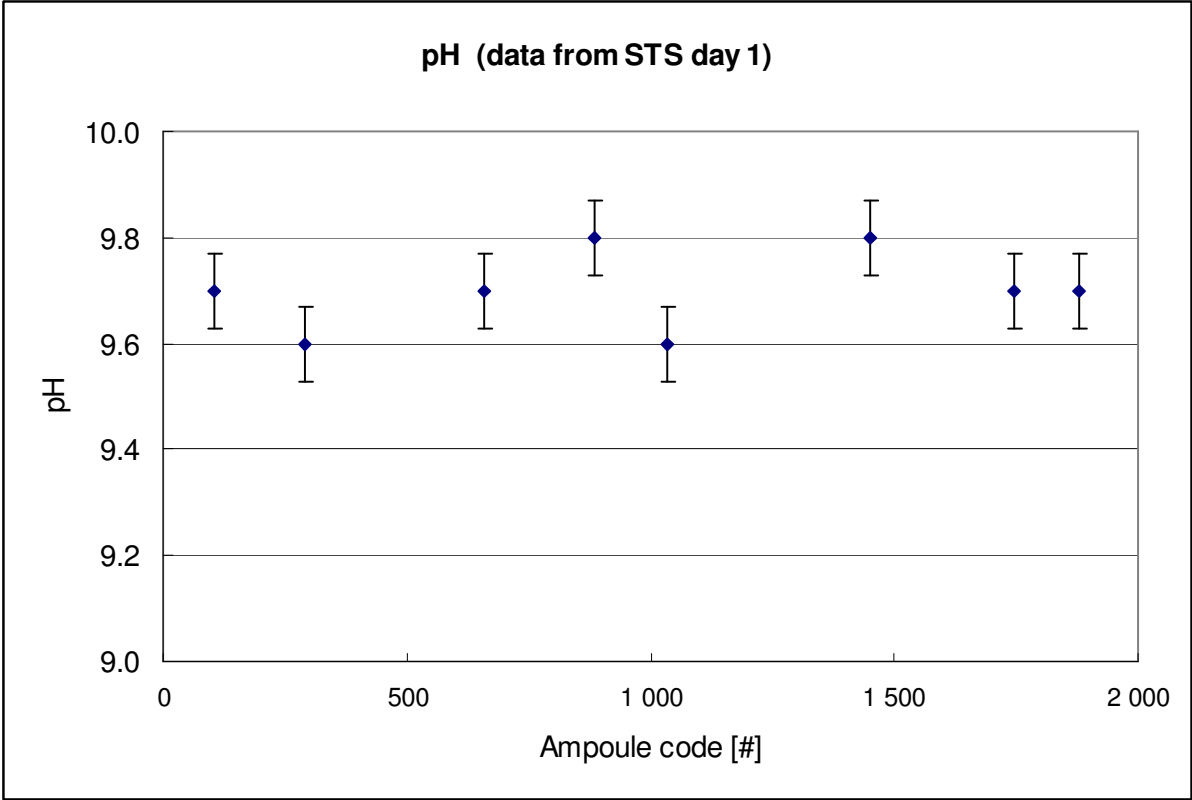
Annex D: Graphical and tabular presentation of the results of the characterisation studies

Annex A: Results of the homogeneity studies

The graphs show ampoule averages and their 95 % confidence intervals of the mean of two replicates per bottle. Confidence intervals were based on the 'within-bottle' standard deviation for each method rather than on the standard deviation of the replicates per ampoule. Absolute values do not necessarily agree with the certified values due to potential laboratory bias, which is irrelevant for the evaluation of homogeneity.



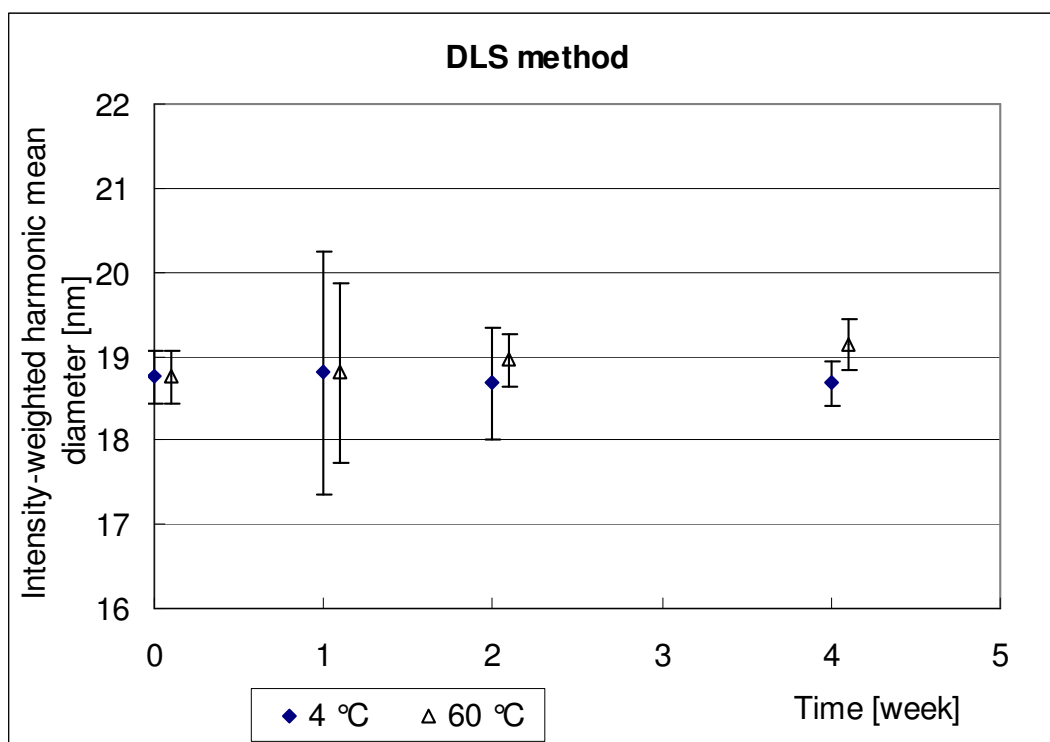


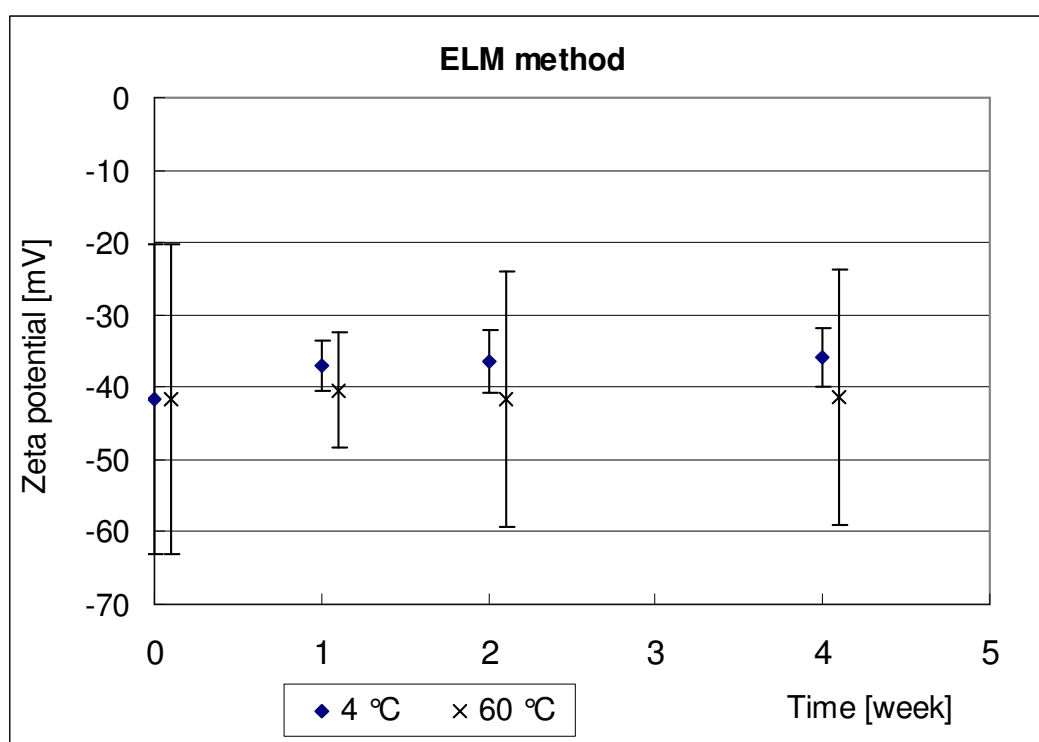
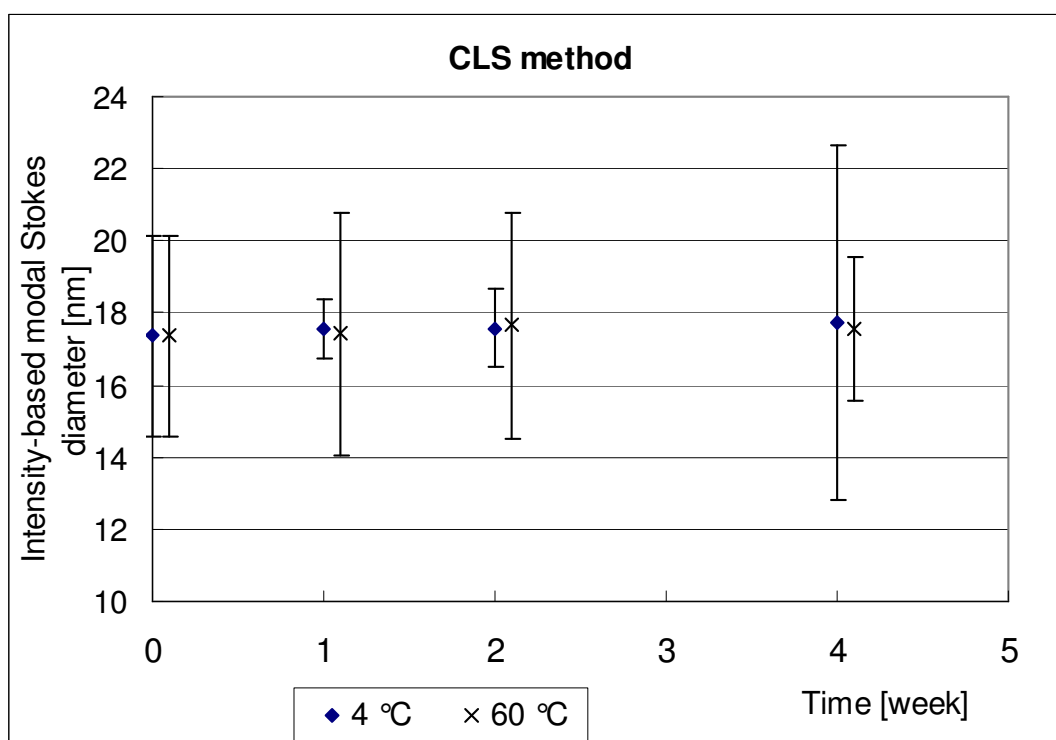


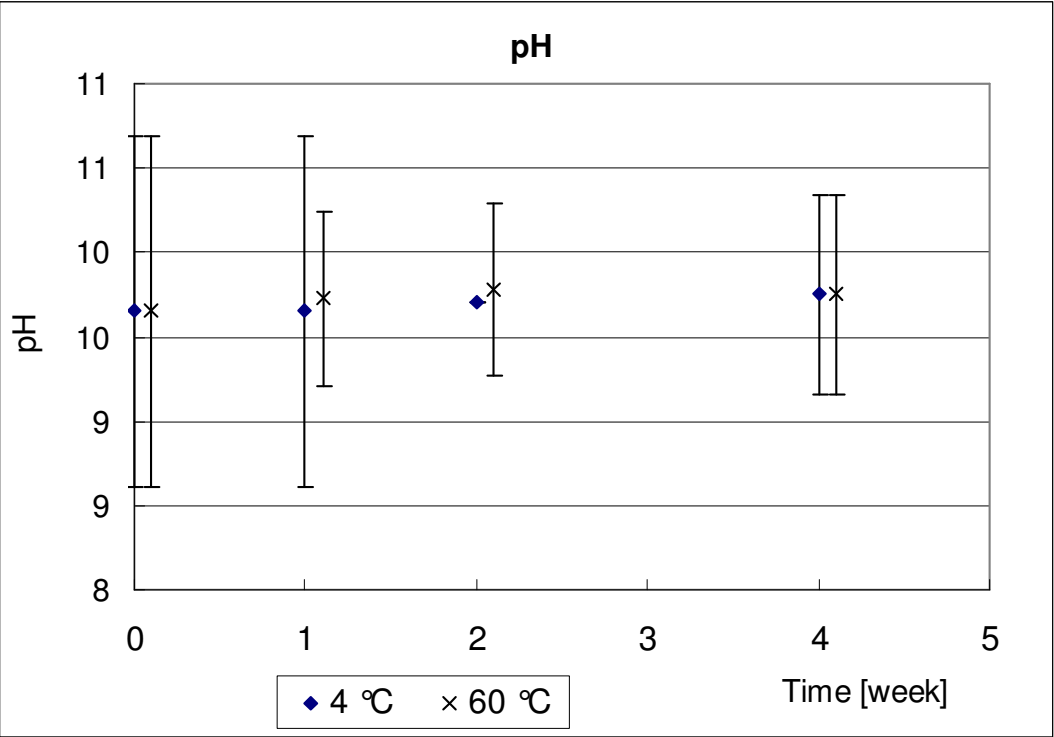
Annex B: Results of the stability studies

Short-term stability graphs

The graphs show averages per time point and their 95 % confidence intervals of the mean of the four replicates per time. Confidence intervals are based on the standard deviations of the four replicates per time/temperature combination. Data for different temperatures were measured at the same time but are graphically separated to make the graphs easier to read. Only one set of samples per method was measured for $t = 0$, which was entered twice in the graphs. Absolute values do not necessarily agree with the certified values due to potential laboratory bias, which is irrelevant for the evaluation of homogeneity.

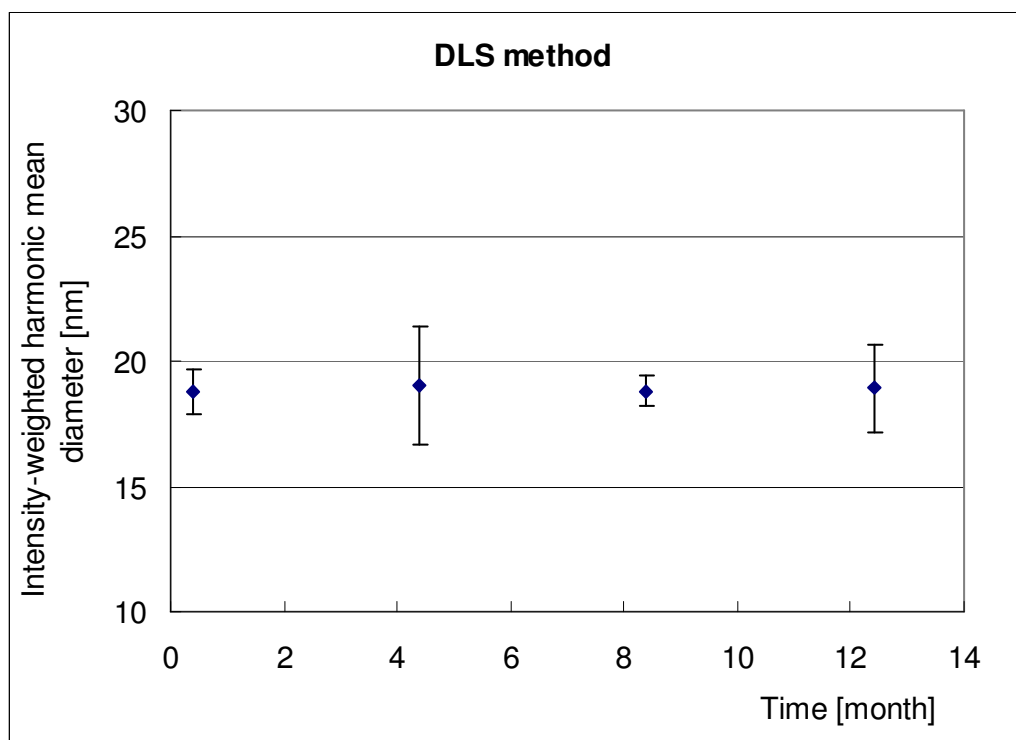


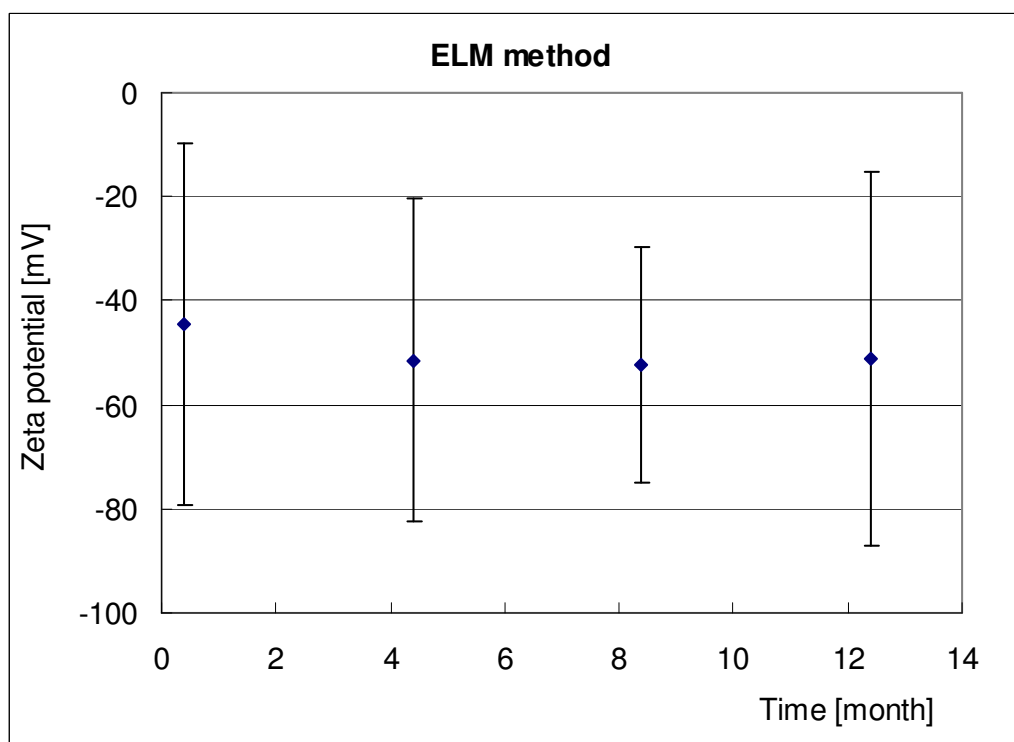
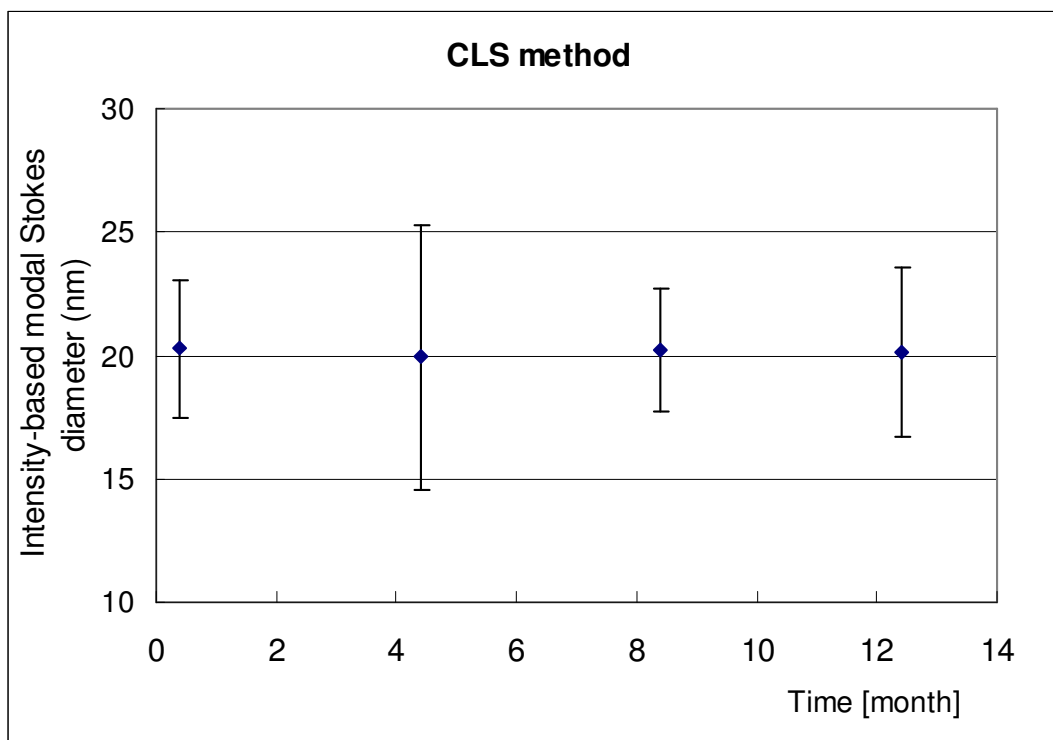




Long-term stability graphs

The graphs show averages per time point and their 95 % confidence intervals of the mean of the 16 replicates per time. Confidence intervals are based on the standard deviations of the 16 replicates per time/temperature combination. Absolute values do not necessarily agree with the certified values due to potential laboratory bias, which is irrelevant for the evaluation of homogeneity.





Annex C: Instruments and methods used for the characterisation

DLS instruments and methods

Lab code	Instrument details	Analysis type/data interpretation	Scattering angle (°)	Wavelength of laser (nm)	Reported distribution form	Sample preparation	Sample volume intake (mL)	Temperature (°C)	Dynamic viscosity (mPa·s)	Refractive index SiO ₂ /water	Polidispersity index
5	Particle Sizing Systems, Nicomp DLS	cumulants	90	632.8	intensity	measured as received	0.7	23	0.932	n.a.(*)/1.33	0.1
6	Beckman Coulter, Nanosizer N 4 Plus	cumulants	90	632.8	Intensity	measured as received	2.5	25	0.89	n.a.(*)/1.33	0.2
10b	Horiba, LB-550	frequency	177	650	intensity	measured as received	4	24	0.8949–0.9566	n.a.(*)/1.33	n.a (*)
10a	Malvern, Zetasizer Nano ZS	cumulants	173	632.8	intensity	measured as received	4	25	0.9	n.a.(*)/1.33	0.1
11	Malvern, Zetasizer Nano ZS	cumulants	173	633	intensity and volume	measured as received	2.5	25	0.8872	n.a.(*)	0.1
12	ALV, CGS-3	cumulants	90	632.8	Intensity	measured as received	1.5	21.0–21.6	0.9640–0.9776	n.a.(*)/1.33	0.1
13	Microtrac, Nanotrac	frequency	180	780	intensity and volume	measured as received	2.5	20.8–24.2	0.9–1.0	1.46/n.a.(*)	0.1
14	Horiba, LB-550	frequency	180	650	intensity and volume	measured as received	3.5	20	1	1.46/n.a.(*)	n.a (*)
15	Sympatec, Nanophox	cumulants	90	632.8	intensity and volume	measured as received	2	20.0–20.1	1	n.a.(*)/1.33	0.1
16	Malvern, Zetasizer Nano ZS	cumulants	173	633	intensity and volume	measured as received	1	25	0.8872	1.46/1.33	0.1

17a	Malvern, HPPS	cumulants	173	632.8	intensity and volume	measured as received	2.5	25	0.89	1.46/n.a.(*)	0.03
17b	Sympatec, Nanophox	cumulants	90	632.8	intensity and volume	measured as received	2.5	25	0.89	1.46/n.a.(*)	0.04
18a	Malvern, Zetasizer Nano ZS	cumulants	173	632.8	intensity and volume	measured as received	1.5	25	0.89	1.46/1.33	0.1
18b	Sympatec, Nanophox	cumulants	90	632.8	intensity and volume	measured as received	1.5	25	0.89	1.46/1.33	0.04
19	Malvern, Zetasizer Nano ZS	cumulants	173	632.8	intensity and volume	measured as received	1.5	25	0.8872	1.46/1.33	0.1
20	Precision Detectors, PDEXPERT	cumulants	90	658	intensity	measured as received	0.5	24.4	0.9	n.a.(*)/1.3	0.3
21	Malvern, Zetasizer Nano	cumulants	173	633	intensity and volume	measured as received	1.5	25	0.886	n.a.(*)/1.33	0.1
22	Malvern, Zetasizer Nano ZS	cumulants	173	633	intensity and volume	measured as received	0.1	25	0.89	n.a.(*)/1.33	0.1
23	Horiba instruments,	frequency	180	650	intensity and volume	measured as received	3	21.8–23.1	0.96	1.46/n.a.(*)	0.1
24	Malvern, Zetasizer Nano ZS	cumulants	173	633	intensity and volume	measured as received	2.5	25	0.8872	n.a.(*)/1.33	0.1
25	Sympatec, Nanophox	cumulants	90	632.8	intensity and volume	measured as received	2.5	25	0.89	1.46/1.33	0.04
26	Malvern, Zetasizer Nano ZS	cumulants	173	633	intensity and volume	measured as received	1	25	0.8872	1.46/n.a.(*)	0.1
27	Malvern, Zetasizer Nano ZS	cumulants	173	633	intensity and volume	measured as received	0.4	25	0.887	1.46/n.a.(*)	0.1

(*) n.a. value not reported from the laboratory

CLS instruments and methods

Lab code	Instrument properties					Sample preparation	Calibrant			Sucrose solution		Lab temp. (°C)
	Instrument details	Analysis type	Rotational speed (rpm)	Laser wavelength (nm)	Reported distribution form		Type/ manufacturer	Density (g/cm ³)	Certified value (nm ₂)	Low density (m/m)	High density (m/m)	
1	LUMiSizer 611, LUM GmbH	homogeneous	4 000	470	intensity and volume	measured as received	–	–	–	–	–	–
2	XLI ProteomeLab, Beckman	homogeneous	8 000	635	volume, no intensity	measured as received	–	–	–	–	–	23
3	DC24000, CPS Instruments	line-start	24 000	405	intensity and volume	measured as received	PVC calibration standard, CPS Instruments Inc.	1.385	460 ± 18.85	8	24	23
4	DC20000, CPS Instruments	line-start	20 000	470	intensity and volume	measured as received	PVC calibration standard, CPS Instruments Inc.	1.385	377	8	24	21
5	DC24000, CPS Instruments	line-start	24 000	470	intensity and volume	measured as received	PVC calibration standard	1.385	377 ± 12	0	8	23
6	CPS DC24000, L.O.T Oriel GmbH	line-start	24 000	405	intensity and volume	measured as received	Duke scientific	1.83	490 ± 20	8	24	23
7	LUMiSizer 6110-19, L.U.M. GmbH	homogeneous	4 000	470	intensity and volume	measured as received	–	–	–	–	–	–
8	Optima XL-1 AUC, Beckman Coulter, Palo Alto	homogeneous	10 000/ 20 000	675	intensity and volume	measured as received	–	–	–	–	–	25
9	DC24000, CPS Instruments	line-start	24 000	470	intensity and volume	measured as received	Duke scientific	1.05	300 ± 5.1	3	13	20

10	DC20000, CPS Instruments	line-start	20 000	405	intensity and volume	measured as received	PVC calibration standard, CPS Instruments Inc.	1.385	460 ± 20	2	8	20
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Electron microscopy- SEM/TEM instruments

Lab code	Method/sample volume intake	Instrument/software	Sample preparation/drying	Sample grid/sample holder/frame size	Calibration	Particles' count	Ampoules storage temp. (prior to analysis) (°C)
3	TEM: micropipette 50 µL	FEI Tecnai 10	no dilution/1hour at room temp. in a laboratory hood	Copper grid with carbon layer (300 mesh)	Last PQ on 26.10.2009 with SiO ₂ particles	500	20
3a	SEM: micropipette 50 µL	Jeol 7500F/ Software SmileView 2.2 and Excel 2003	no dilution/1hour at room temp. in a laboratory hood	Golden silicium substrate (25 mm ²), frame size 1280*1024 pixels	Last PQ on 15.5.2009 on Au particles	500	20
5	TEM: 5 µL	Philips CM200 STEM/ iTEM by Olympus Soft Imaging Solutions	no dilution/ under vacuum conditions during 2 minutes	Cu-grids, 200 mesh, carbon coated, low background holder; frame size 870 x 696 nm	Last PQ 06.05.2010 on grating replica	> 500	22
Laboratory comment:		In the iTEM-software the particles were measured as a circle, so it was not possible to give a mean aspect ratio.					
11	TEM: 3 µL	Tecnai G2 20 S-TWIN, FEI/- Image J, Origin	volume ratio sample:wate = 1:10 (v/v),diluted with ultrapure water and additional filtration with 0.1µm filter size, 3 hours at room temp.	copper grid, carbon film, diameter of the frame 3 mm.	n.a. (*)	500	21
11a	SEM: 3 µL	Hitachi S-4800/ Image J, excel		silicon chip , 4 × 4 mm	n.a. (*)	500	21
15	TEM: 1 mL	FETEM/JEOL JEM-2100F UHR/ Gatan Digital	no dilution/over 4 hours and dried in digital dry cabinet	JEOL Double tilt holder (EM-31640), Lacey Formar/Carbon 200mesh Copper grid (Ted Pella	SPI supplies, Lot 1081229 No.835 (99 ± 1.118 nm)/ Last PQ 4.03.2010	> 500	19±1

		Micrograph		No.01881-F),1 024 x 1 024 Pixels			
15a	SEM: 1 mL	FESEM/JEOL JSM-6500F/ Gatan Digital Micrograph	dilution 1:50 (v/v) over 4 hours and dried in digital dry cabinet	JEOL SEM holder, 1 drop on copper (QCM) ,1drop on wafer (FD100),1280 x 1027 Pixels	Agar (457.8 ± 2.02 nm)/ Last PQ 18.03.2010 on Agar S170A	500	19±1
Laboratory comment:		The measurement of ERM-FD100 was performed only by picking particles with the diameter from 10 nm to 30 nm and for QCM only from 20 nm to 50 nm to avoid data from particles overlapping.					
16	TEM: 5 µL	TEM Jeol 2100/ ImageJ	dilutions to a target mass fraction of 0.075/1 hour in cabinet with nitrogen atmosphere	EM-11210SQCH Specimen Quick-change holder, 400 Mesh Cu pre coated with lacey/thin carbon film (Pacific Grid Tech)	NIST 8013 (56 ± 0.5 nm)/ Last PQ on 17.11.2009 on MagICal s/n 988	> 500	20
20	TEM: 2.5 µL	2000FX JEOL/ ImageJ	dilution 1:50 (v/v) 30 minutes in laboratory bench	JEOL single tilt specimen holder EM-SQH10, carbon coated TEM grids	Agar S106 (463 ± 1 nm)/ Last PQ 03.06.2010 on 2160 lines/mm grating and Catalase crystals.	> 500	18-22
33	TEM: 10 µL	Jeol JEM 1011/ standard software JEOL JEM 1011	no dilution/ excess sample removed, grid air dried at room temp. for 15 min	standard holder of Jeol JEM 1011, Formvar/ carbon 400 mesh copper from EMS	NIST RM 8012 (27.6 ± 2.1 nm)/Last PQ 19.05.2010 on Grating grid (Pelco 2160 lines/mm) and TMV virus (width 18 nm)	> 500	21
Laboratory comment:		Particle sizing was done manually with the software of the JEOL JEM 1011. No automated particle sizing software was used.					
34	TEM: Thies- Weesie dip method	Philips 120CM TEM/ImageJ	no dilution/five minutes on filter paper in a clean room	Carbon coated 200 mesh copper grid	Norrox Scientific Ltd. No. 695 (108.5 ± 2 nm)/ Last PQ 14.4.2010 - 26.4.2010 on MAG*1*CAL	> 500	23

(*) n.a. value not reported from the laboratory

SAXS instruments

Lab code	Instrument details	Electric current [mA]	Type of X-ray	Scattering angular range/resolution	Number of size class	Collimation type	Sample preparation	Sample volume (mL)	Reported distribution form	Mathematical fitting models	Temp. (°C)
28	BESSY II, HZB SAXS at PTB FCM	150-300	0.155 nm (8 000 eV)	type 2°/0.0015°	n.a.(*)	point	measured as received	0,02	intensity and volume	Gaussian distribution	RT
29	PANalytical B.V., vacuum SAXS camera, Expert SAXS	40	1.54178 nm	0.1-6; 0.05 degrees	100 channels, fit by analytical function	line	measured as received	0.1	intensity and volume	Gaussian distribution	25
30	Anton Paar, SAXSess (Kratky Type)	40	0.1542 nm	0.08–6.0 nm ⁻¹ /0,002 nm ⁻¹	n.a.(*)	line	measured as received	0.04	intensity and volume	Polydisperse gauss sphere	25
31	Anton Paar, SAXSess	50	0.1542 nm	0.07–6.3 nm	30-80 nm in steps of 0.7 nm	line	measured as received	0.1	intensity and volume	Inverse RDG transform	25
32	Rigaku, small angle X-ray scattering	0.66	0.1542nm	0.067–5 nm ⁻¹	n.a.(*)	point	measured as received	0.000385	intensity	Guinier	21.7

(*) n.a. value not reported from the laboratory

ELM instruments

Lab code	Instrument details	Laser power (mV)	Wavelength of laser (nm)	Detector type	Detector scattering angle (°)	Sample preparation	Temperature (°C)	Dynamic viscosity (mPa·s)
5	Malvern, Zetasizer Nano ZS	4	633	APD	13	measured as received	25	0.8872
10	Malvern, Zetasizer Nano ZS	4	633	APD	173	measured as received	25	0.8865-0.8879
11	Malvern, Zetasizer Nano ZS	4	633	APD	13	measured as received	–	–
13	Particle Metrix GmbH, ZetaView PMX 100, S/N 117	5	650	Video camera	90	measured as received	21.7–25.5	0.8810–0.9500
16	Malvern, Zetasizer Nano ZS	4	633	APD	173	measured as received	25	0.8904
19	Malvern, Zetasizer Nano ZS, MAL 1029404	4	632.8	APD	12.8	measured as received	25	0.8872
21	Malvern, Zetasizer 3000 HS	5	633	APD	12.8	measured as received	25	0.89
22	Malvern, Zetasizer Nano ZS	4	633	APD	173	measured as received	25	0.8904
26	Malvern, Zetasizer Nano ZS	4	633	APD	173	measured as received	25	0.8872
27	Malvern, Zetasizer Nano ZS	4	633	APD	173	measured as received	25	0.8862–0.8883

pH instruments

Lab code	Instrument details	Instrument type and number	Type of electrode	Calibration range	Standard or reference material used for calibration
5	Horiba	Twin pH-meter B213	Glass-electrode	pH 4 and pH 10	Titrisol 9884,1.09887,9890
10	Metrohm Switzerland	1,7440010/18441	Glass-electrode	pH 4 and pH 9	Buffer Solution pH 7
11	METTLER TOLEDO	FE20	LE438	pH 4 and pH 6.86	Potassium Hydrogen Phthalate (pH 4.00) and Mixed Phosphate (pH 6.86)
13	WTW	PH/cond 340i	SenTix 81 Platin Ceramics Glass membrane	pH 4 and pH 7	DM Messtechnik buffer solutions
16	Cole-Parmer OR Malvern Zetasizer	pH 500 series	n.a.(*)	pH 7 and pH 10	Oakton buffers, (pH 7) and (pH 10)
19	Oaklon Instruments	pHTestr	Double Junction	pH 4 and pH 10	Buffer solutions, pH = 4, 7, 10
21	Thermo Electron Corporation	Orion 3 STAR	InLab@Semi-micro, Mettler Toledo	pH 4 and pH 12	Buffer Solution pH 7
22	Thermo	Orion 410A+	Ag/AgCl	pH 4, pH 7and pH 10	Fisher Sci Buffer Solution pH 4, 7, and 10
26	Accumet	AR25	Glass combination	pH 2 and pH 10.01	Buffers, pH 2, 4.01, 7.01 & 10.01
27	Shindengen	ISFET pH meter KS723	ISFET	pH 1 and pH 14	Commercial buffers provided with electrode

(*) n.a. value not reported from the laboratory

Annex D: Graphical and tabular presentation of the results of the characterisation studies

The graphs show expanded uncertainties as reported from the laboratories, not standard deviations. Results with a low standard deviation may well have a large uncertainty. Laboratories 11 and 12 did not report any uncertainties for the DLS results. Their uncertainty was estimated and given as the confidence interval (CI) of a mean for 95 % probability and degrees of freedom (n-1) (Section 6.3.5, eq. 2).

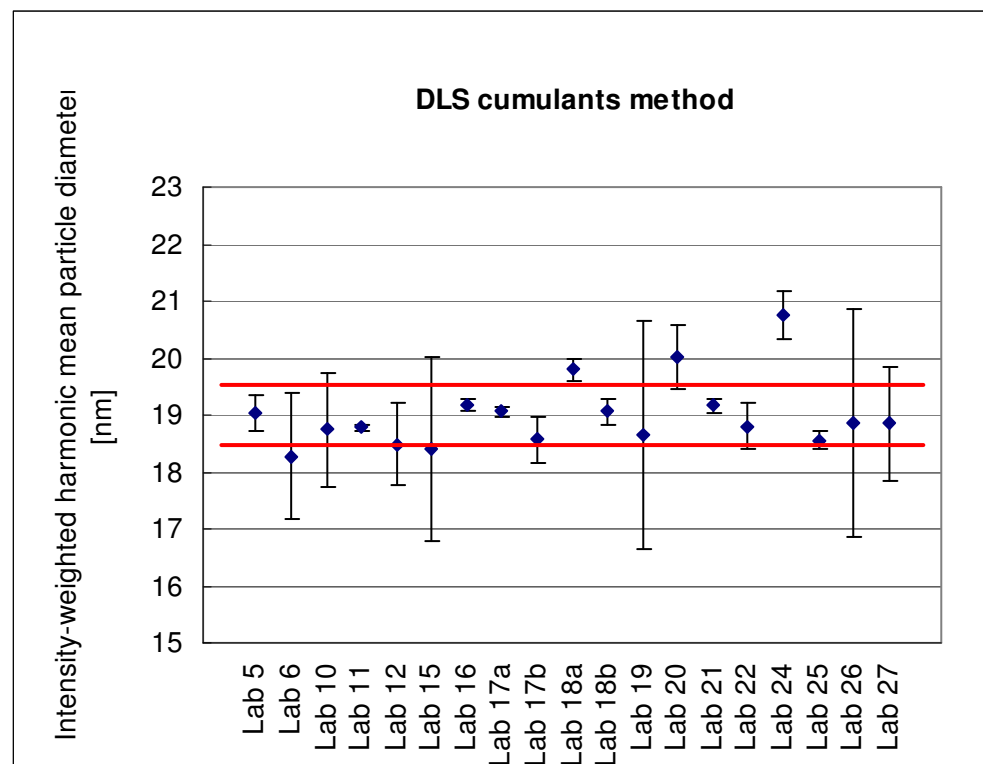
The solid red lines in the graphs mark the intervals of the certified values obtained with each method. This is the certified value \pm expanded uncertainty ($k = 2$) estimated as described in Section 7.1.

Certified values

Intensity-weighted harmonic mean particle diameter (DLS cumulants method)

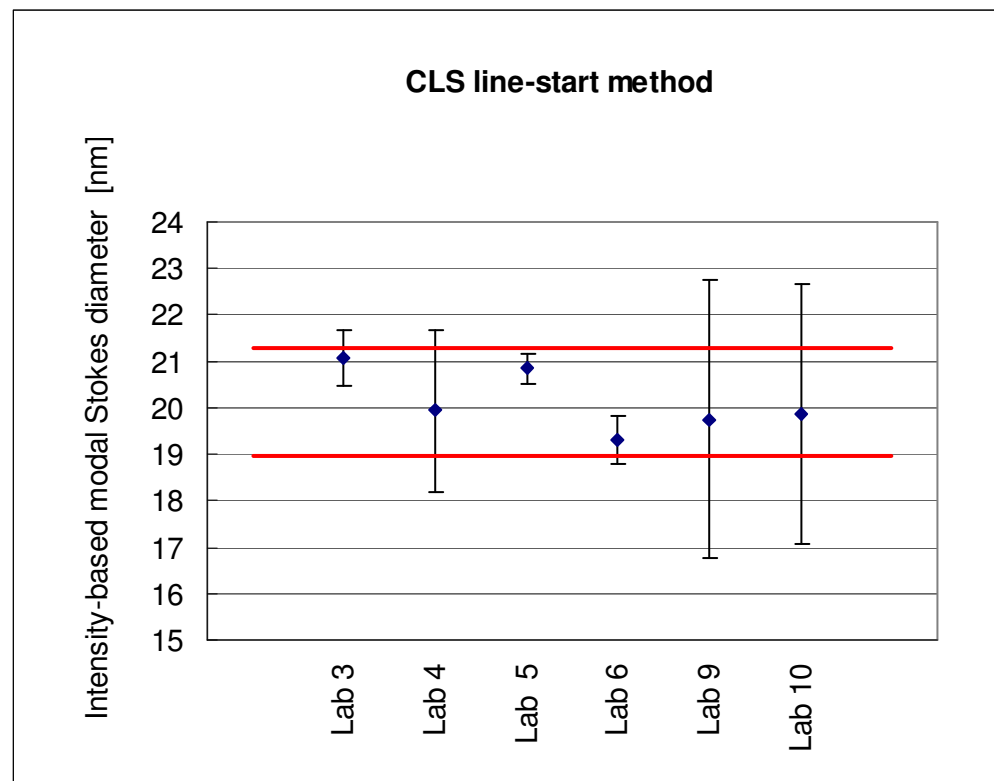
Laboratory code	Average	Expanded uncertainty ($k = 2$)
[#]	[nm]	[nm]
Lab 5	19.03	0.33
Lab 6	18.28	1.10
Lab 10	18.74	1.00
Lab 11	18.78	0.05
Lab 12	18.49	0.73
Lab 15	18.41	1.60
Lab 16	19.17	0.10
Lab 17a	19.06	0.10

Lab 17b	18.57	0.40
Lab 18a	19.79	0.20
Lab 18b	19.06	0.23
Lab 19	18.65	2.00
Lab 20	20.01	0.56
Lab 21	19.16	0.12
Lab 22	18.80	0.40
Lab 24	20.76	0.40
Lab 25	18.56	0.20
Lab 26	18.87	2.00
Lab 27	18.85	1.00



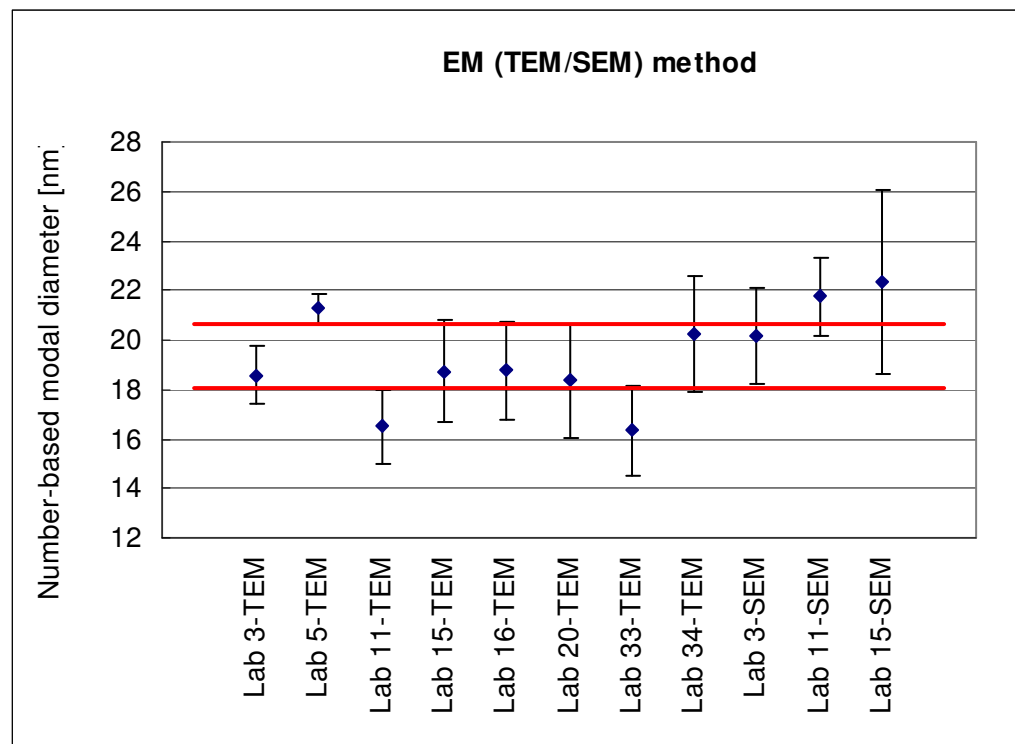
Intensity-based modal Stokes particle diameter (CLS line-start method)

Laboratory code	Average	Expanded uncertainty ($k = 2$)
[#]	[nm]	[nm]
Lab 3	21.07	0.61
Lab 4	19.94	1.74
Lab 5	20.84	0.32
Lab 6	19.31	0.51
Lab 9	19.76	3.00
Lab 10	19.88	2.80



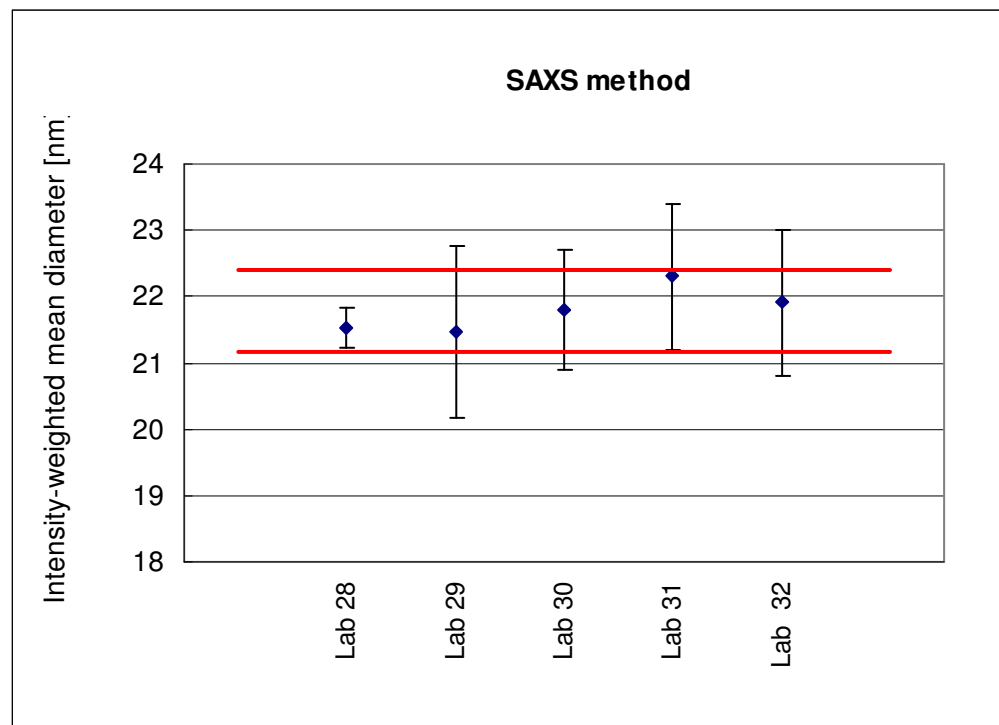
Number-based modal particle diameter (TEM/SEM)

Laboratory code	Average	Expanded uncertainty ($k = 2$)
[#]	[nm]	[nm]
Lab 3	18.57	1.17
Lab 5	21.26	0.60
Lab 11	16.49	1.46
Lab 15	18.74	2.07
Lab 16	18.75	2.00
Lab 20	18.38	2.30
Lab 33	16.34	1.84
Lab 34	20.24	2.34
Lab 3a	20.16	1.90
Lab 11a	21.74	1.60
Lab 15a	22.36	3.70



Intensity-weighted mean particle diameter (SAXS)

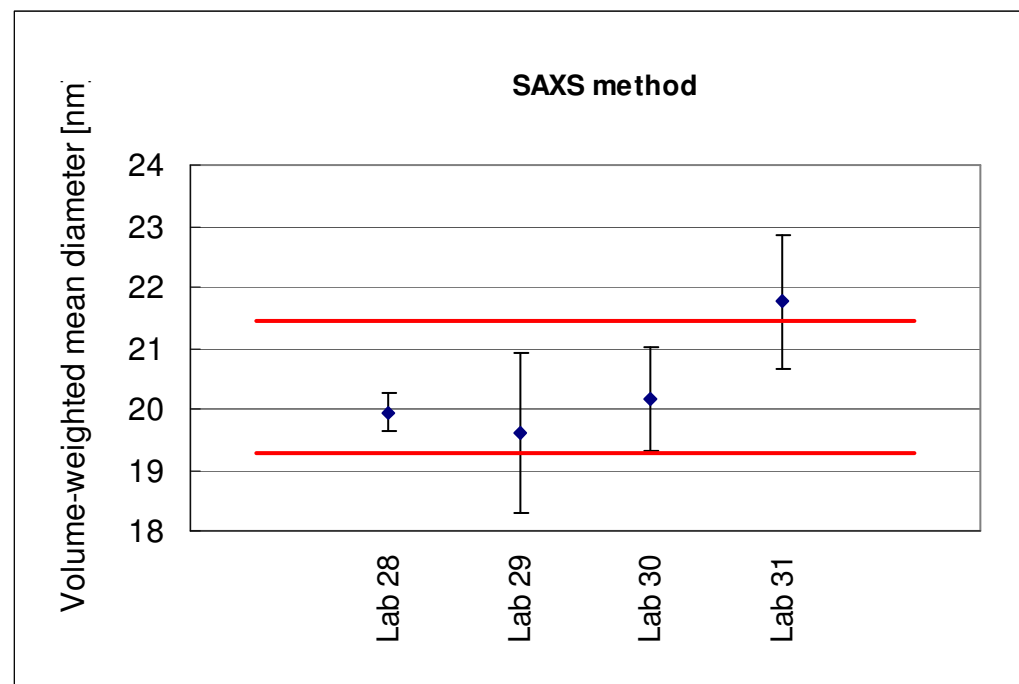
Laboratory code	Average	Expanded uncertainty ($k = 2$)
[#]	[nm]	[nm]
Lab 28	21.52	0.30
Lab 29	21.46	1.30
Lab 30	21.80	0.90
Lab 31	22.31	1.10
Lab 32	21.91	1.10



Indicative values

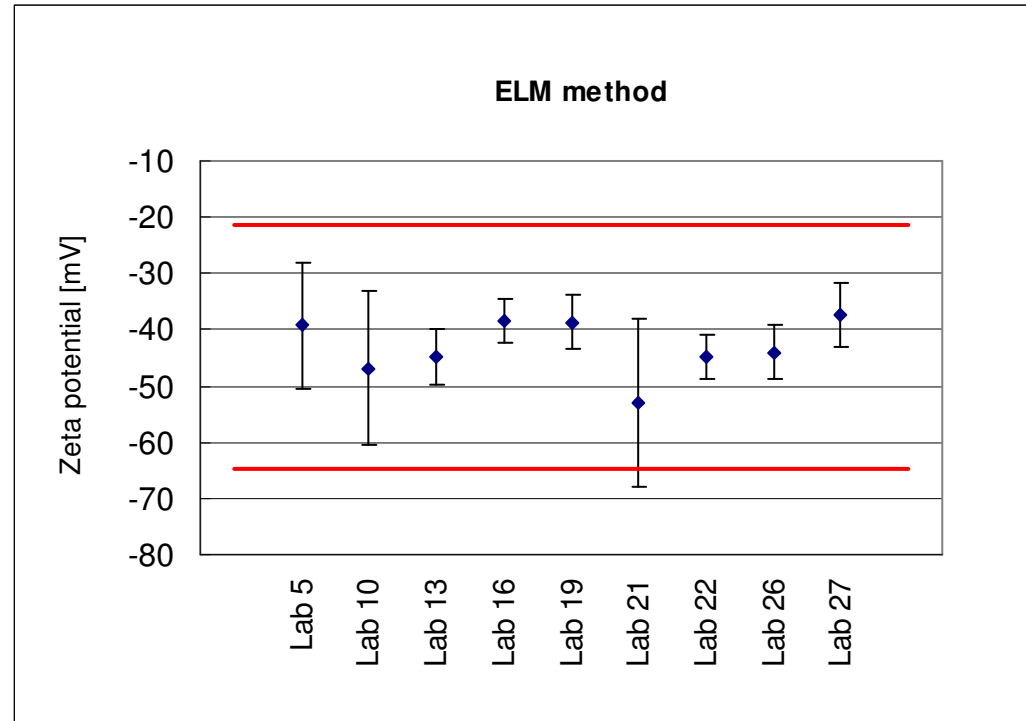
Volume-weighted mean particle diameter (SAXS)

Laboratory code	Average	Expanded uncertainty ($k = 2$)
[#]	[nm]	[nm]
Lab 28	19.95	0.30
Lab 29	19.60	1.30
Lab 30	20.17	0.86
Lab 31	21.76	1.10



Zeta potential (ELM)

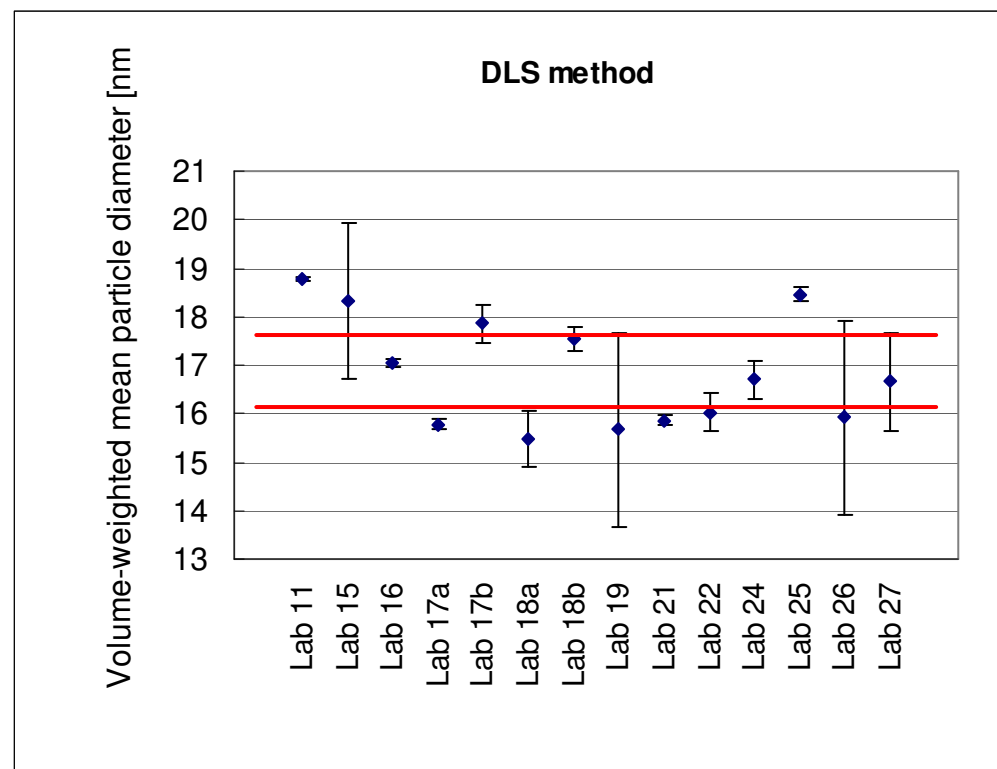
Laboratory code	Average	Expanded uncertainty ($k = 2$)
[#]	[nm]	[nm]
Lab 5	-39.27	11.11
Lab 10	-46.79	13.81
Lab 13	-44.76	5.00
Lab 16	-38.42	3.97
Lab 19	-38.63	4.80
Lab 21	-53.11	14.90
Lab 22	-44.74	4.00
Lab 26	-44.03	4.80
Lab 27	-37.38	5.83



Additional material information

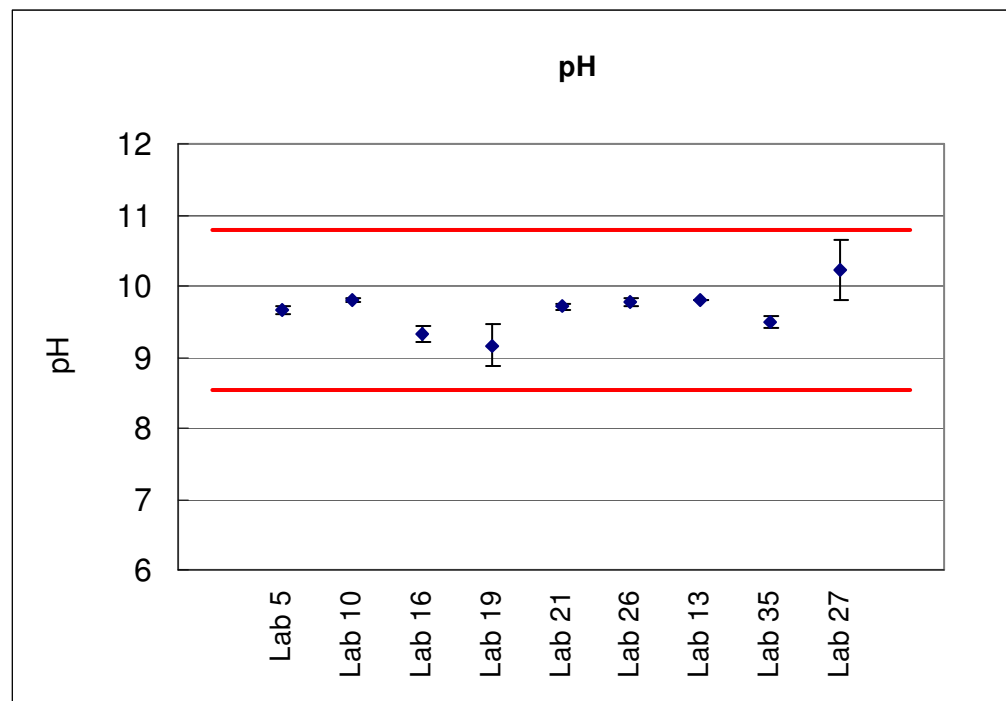
Volume-weighted mean particle diameter (DLS method)

Laboratory code	Average	Expanded uncertainty ($k = 2$)
[#]	[nm]	[nm]
Lab 11	18.78	0.05
Lab 15	18.31	1.60
Lab 16	17.04	0.10
Lab 17a	15.78	0.10
Lab 17b	17.86	0.40
Lab 18a	15.46	0.57
Lab 18b	17.54	0.24
Lab 19	15.67	2.00
Lab 21	15.86	0.10
Lab 22	16.02	0.40
Lab 24	16.70	0.40
Lab 25	18.45	0.15
Lab 26	15.92	2.00
Lab 27	16.65	1.00



pH

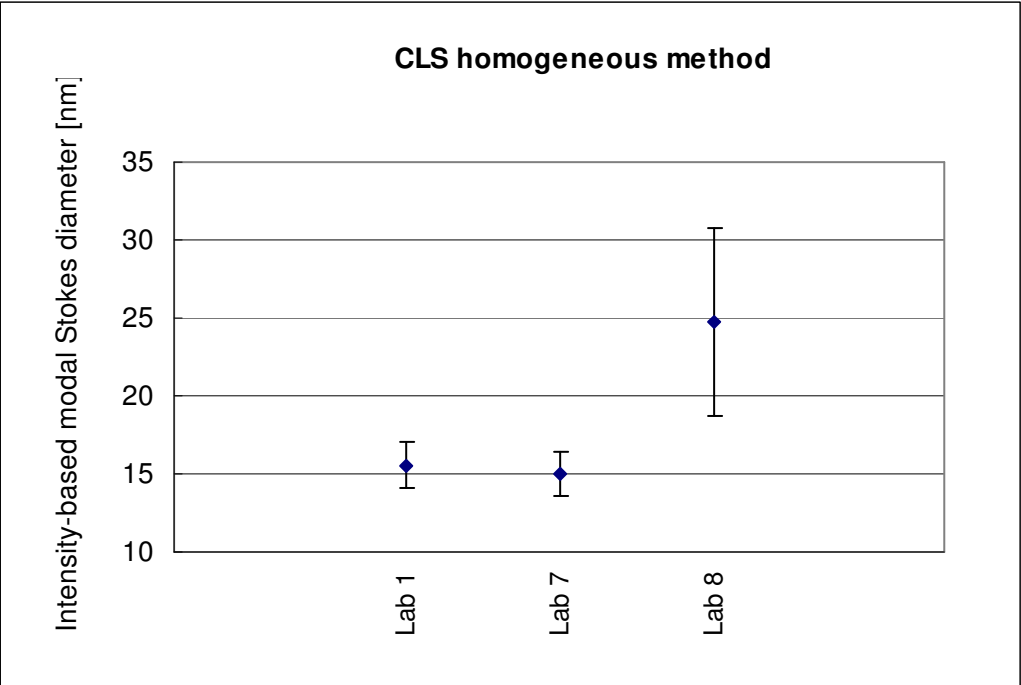
Laboratory code	Average	s
[#]		
Lab 5	9.7	0.05
Lab 10	9.8	0.03
Lab 16	9.3	0.12
Lab 19	9.2	0.29
Lab 21	9.7	0.04
Lab 26	9.8	0.05
Lab 13	9.8	0.01
Lab 35	9.5	0.09
Lab 27	10.2	0.42



Additional material information

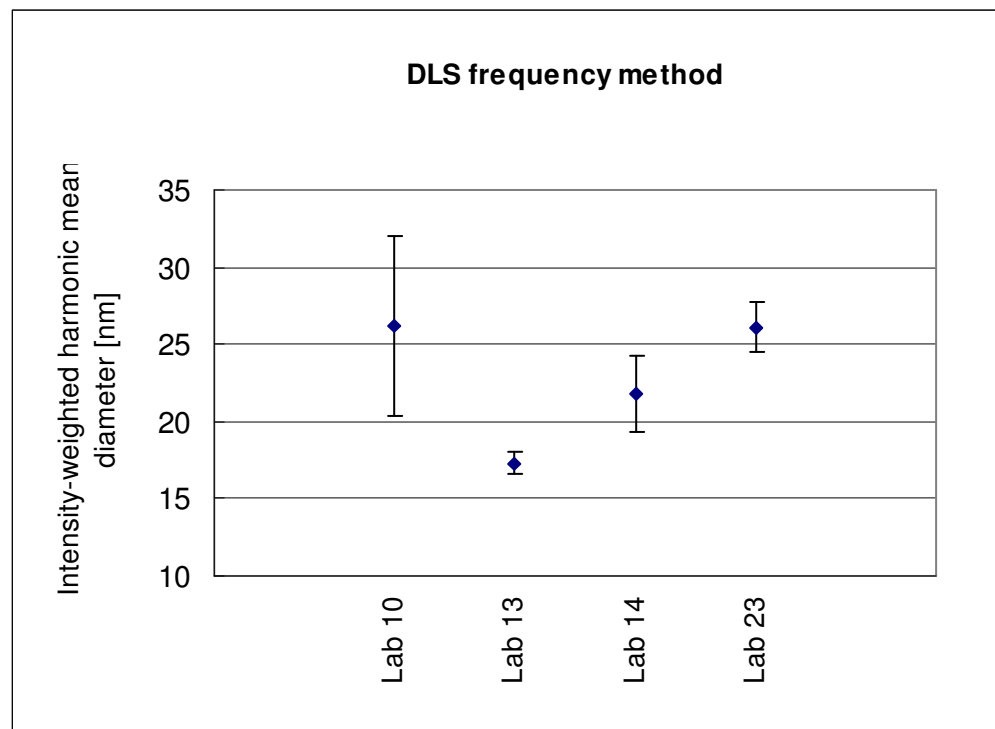
Intensity-based particle diameter (CLS homogeneous method)

Laboratory	Average	Expanded uncertainty ($k = 2$)
[#]	[nm]	[nm]
Lab 1	15.53	1.46
Lab 7	14.98	1.40
Lab 8	24.76	6.00



Intensity-weighted harmonic mean particle diameter (DLS frequency method)

Laboratory	Average	Expanded uncertainty ($k = 2$)
[#]	[nm]	[nm]
Lab 10	26.21	5.80
Lab 13	17.27	0.70
Lab 14	21.78	2.50
Lab 23	26.09	1.60



European Commission

EUR 24620 EN – Joint Research Centre – Institute for Reference Materials and Measurements

Title: Certification of Equivalent Spherical Diameters of Silica Nanoparticles in Water , Certified Reference Material ERM[®]-FD100

Author(s): A. Braun, K. Franks, V. Kestens, G. Roebben, A. Lamberty, T. Linsinger

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Abstract

This report describes the certification of the equivalent spherical diameters of silica nanoparticles suspended in aqueous solution, Certified Reference Material (CRM) ERM-FD100[®]. The CRM has been certified by the European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE.

The intended use of this ERM-FD100 is to check the performance of instruments and methods that determine the particle diameter of nanoparticles (particle size ranging from approximately 1 nm to approximately 100 nm) suspended in a liquid medium. It is available in 10 mL pre-scored amber glass ampoules containing approximately 9 mL of suspension.

The CRM was prepared from commercially available colloidal silica (Koestrosol 1530, Chemiewerk Bad Koestritz GmbH, DE).

Certification of the CRM included testing of the homogeneity and stability of the ampouled diluted raw material, as well as the characterisation using an intercomparison approach.

The material has been certified for the equivalent diameter of the silica nanoparticles in aqueous suspension using different methods. Certified values are the cumulants dynamic light scattering (DLS) intensity-weighted harmonic mean particle diameter, the line-start centrifugal liquid sedimentation (CLS) intensity-based modal (Stokes) particle diameter, the electron microscopic (transmission electron microscopy (TEM)/ scanning electron microscopy (SEM)) number-based modal particle diameter and the small angle X-ray scattering (SAXS) intensity-weighted average particle diameter.

Indicative values have been established for the volume-weighted mean equivalent spherical diameter via the SAXS method and for the zeta potential via the electrophoretic mobility (ELM) method. Additional informational values are given for the volume-weighted mean diameter via the DLS method, and the pH value of the ERM-FD100 suspension.

Uncertainties are expanded uncertainties estimated in accordance with the Guide to the expression of uncertainty in measurement (GUM) with a coverage factor of $k = 2$, corresponding to a confidence interval of about 95 %. An exception is the mean equivalent volume-weighted diameter determined by the SAXS method which has a coverage factor of 2.8..

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